Full Measure Industries, LLC

October 30, 2017

Devon Pattillo
Agricultural Marketing Specialist
National Organic Program
1400 Independence Avenue, SW
Room 2646-S, STOP 0268
Washington, DC 20250-0268

Dear Mr. Pattillo:

Thank you very much for your very prompt response to our application to have calcium acetate included on the National List and for the list of suggested revisions. The revised petition and copies of our product label are included. We appreciate your help with this matter and look forward to hearing from you.

Sincerely,

Raymond B. Frizzell
President

11 Broadcommon Road, Unit 2, #400 • Bristol, Rhode Island 02809 • Phone 401.447.9479
Patent # US7695541B1 • Made in the USA

NOP Petition for Inclusion of Calcium Acetate in National Organic List of Substances Allowed

Item A.1
Full Measure Industries, LLC is petitioning to have calcium acetate included in the National Organic List within the following category: § 205.601 Synthetic substances allowed for use in organic crop production.

Item A-2
Full Measure Industries, LLC is petitioning to have calcium acetate included in the National List under the category of vitamins and minerals.

Item B.

1.Substance's chemical and material common name:
Calcium acetate.
Synonyms: acetate of lime; calcium ethanoate
CAS #62-54-4.

2. Petitioner and Manufacturer Information

Full Measure Industries, LLC is a family owned business headquartered in Bristol, RI. Raymond Frizzell founded the business and is the President and CEO. His son Nathan serves as Chief of Operations. Full Measure products are currently manufactured by Royal Chemical, a toll blender with manufacturing facilities throughout the US. Our products are currently manufactured at their plant in Chattanooga, TN. Full Measure products are currently distributed by Miller Chemical Company, an agricultural products distributor, whose headquarters are in Hanover, PA. Miller has sales reps throughout North and South America.

Contact Information:
Raymond B. Frizzell
President
FullMeasure Industries, LLC
11 Broadcommon Road
Unit 2, #400
Bristol, RI 02809
401 447 9479
ray@fullmeasurecal.com

3. Intended or current use of substance
Calcium acetate is a component of two Full Measure products. One is used as a soil amendment, a plant micronutrient, and a soil pH adjuster. The second is used as a spray which prevents sunscald, as a film to lower soil temperature beneath black plastic, and as a temporary covering on greenhouse glass to lower temperatures in the greenhouse.

4. Intended Activities and Application Rate

These products have been used on beans, soybeans, cotton, tobacco, peppers, melons, peanuts, tomatoes, strawberries, hay, turf grass, citrus, and apples, among other crops.

Products are applied through drip irrigation lines or by spray rig and may be applied directly to soil or to foliage. Products are used to amend soil, adjust pH, and as a plant micronutrient. They are also sprayed on fruit and vegetables to prevent sunscald and lower heat stress. In addition, they are sprayed on black plastic to lower soil temperature beneath the plastic and reduce heat stress on plants and as a temporary coating on greenhouse glass to lower the temperature in the greenhouse.

Products are a thick liquid concentrate. One to two pints of concentrate are mixed with 20 gallons of water for foliar application and applied every 7 to 10 days. For soil application, 2-5 gallons are mixed with 10-20 gallons of water for each acre treated. For sun scald protection or to lower soil temperature under black plastic, 1-2 gallons of product is mixed with 5-20 gallons per acre. Agitation is necessary prior to application to thoroughly mix product and water. Please see product labels for additional information on application.

5. Manufacturing Process

Full Measure Industries' calcium products are made from finely ground limestone which is chelated with acetic acid. During the chelation process, calcium acetate forms and constitutes about 5% of the calcium in the finished product. The rest of the calcium in the product is calcium carbonate.

6. ***Ancillary Substances***

Full Measure products include a surfactant to assist the calcium in staying in solution, a preservative to prevent the growth of mold if the product is stored for long periods of time, and (for our plant nutrient/pH adjuster/soil amendment product) humic acid to provide some additional plant nutrition. The surfactant we are currently using is Xanthan Gum, supplied by C.P. Kelco Corporation of Atlanta. GA. Our humic acid comes from Lignotech Corporation of Rothschild, WI. We are in the process of switching to a preservative with OMRI certification, Biosecure, which is manufactured by Biosecur Lab, Inc of Quebec, Canada. The acetic acid used to chelate the limestone is Glacial Acetic Acid supplied by Univar Corporation of Chattanooga, TN. The limestone we utilize is from a mine in Georgia owned by Imerys Corporation of Raswell, GA and distributed by B.H. Roettker Company of Cincinnatti, OH.

7. A summary of any available previous reviews by State or private certification programs or other organizations.
Not available

8. Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers. If this information does not exist, the petitioner should state so in the petition.

Calcium acetate is registered with the Environmental Protection Agency as a biochemical pesticide used as a yellow jacket attractant by Bull Run Scientific of Richmond, VA. See attached EPA fact sheet (Attachment A) and attached EPA Biopesticides Registration Action Document (Attachment B) for more detail.

Calcium acetate is also on the EPA Safer Chemical Ingredients List. See Attachment D.

9. The Chemical Abstract Service (CAS) number and Product Labels

CAS# 62-54-4.
E263 (food additive number)
Please see attached product labels for Full Measure Industries products.

10. The substance's physical properties and chemical mode of action including (a)Chemical interactions with other substances, especially substances used in organic production;
(b) toxicity and environmental persistence;
9c) environmental impacts from its use and/or manufacture;
(d) effects on human health; and ,
(e) effects on soil organisms, crops, or livestock.

Calcium actetate is a calcium salt of acetic acid. It does occur naturally but is more often manufactured. Calcium acetate can be prepared by soaking calcium carbonate (found in eggshells or in common carbonate rocks, such as limestone or marble, or hydrated lime in vinegar:

$$CaCO_{3(s)} + 2CH_3COOH_{(aq)} \rightarrow Ca(CH_3COO)_{2(aq)} + H_2O_{(l)} + CO_{2(g)}$$
$$Ca(OH)_{2(s)} + 2CH_3COOH_{(aq)} \rightarrow Ca(CH_3COO)_{2(aq)} + 2H_2O_{(l)}$$

Its molecular formula is
$C_4H_8CaO_5$

Its molecular weight is 176.181 g/mol
Please see Attachment C from the Open Chemistry Database for more information on physical and chemical properties.

a.Like all forms of calcium, calcium acetate should not be mixed with phosphates when applying to crops, as it binds phosphates.

b. Low to null toxicity. Breaks down quickly. Calcium acetate is listed on the Environmental Protection Agency's List of Safer Chemicals. Please see Attachment D. The Carcinogenic Potency Project's data suggests it does not cause cancer. Please see attachment E.

c. There is an environmental impact from mining of limestone.

One of our products is currently being tested for possible use in commercial shellfish hatcheries to aid in producing stronger shells in oysters and other commercially grown shellfish under a grant by the state of Rhode Island. Under the same grant, the product is also being tested for use on clam flats that have turned into "ocean deserts" due to acidification. We have also done some preliminary testing ourselves on the possibility of using the product to ameliorate acid rain runoff from roadbeds near sensitive wetlands. All this is evidence of a benign or even beneficial environmental impact.

d. Calcium acetate is consumed orally to treat or prevent calcium deficiency. It is also administered orally to treat hyperphosphatemia in patients with end stage renal disease.

Calcium acetate is also used as a stabilizer and preservative in many food substances under the number E263. It is used as a stabilizer in most hard candies and as an anti-roping agent in most commercially sold breads. It is used in baby food and syrups as a stabilizer. It is used to coagulate soy milk in the commercial manufacture of tofu.

e. Calcium is a necessary building block for cell walls for all living creatures. In addition, calcium aids in the uptake of other micronutrients by plants. A deficit of calcium produces disease in many crops. Examples include hollow heart in potatoes and bitter pit in apples. Plants with high calcium content, and therefore stronger cell walls, are less susceptible to damage by insects and produce fruits and vegetables that have a longer shelf life. Some plants, like peanuts and soybeans, produce fewer "pops" or empty spots in shells when grown with adequate calcium.

Because calcium acetate is highly water soluble, it is immediately available for uptake by plants, unlike many other forms of calcium, which do not become water soluble until exposed to soil microbes and/or acidic conditions. Lime, for instance, may take up to 6 months to become available for uptake by plants. Products that include calcium acetate coupled with less soluble forms of calcium have an immediate impact and an "extended release" effect. For this reason, the inclusion of calcium acetate in the National List has great potential for improving outcomes for organic farming practices.

The agriculture community is becoming increasingly aware of the benefits of using calcium acetate. We are aware of four other calcium acetate products that have recently been added to the market. We have been approached by one of the four largest distributors of agricultural fertilizers and chemicals about producing a private label for them "because we don't yet have a calcium acetate product."

Organic farmers are similarly becoming aware of the benefits of using calcium acetate. We are seeking OMRI certification of our products (and the addition of calcium acetate to the National Organics List as a step toward this) at the urging of our distributor's sales reps who are getting this request from their organic customers, who have learned of our product from their neighbors.

Previous generations of organic farmers made calcium acetate from eggshells and vinegar and appear to have recognized that doing do changed how quickly the calcium from the eggshells was taken up by plants.

11. Safety information about the substance including a Material Safety Data Sheet and a substance report from the National Institute of Environmental Health Studies. If this information does not exist, the petitioner should state so in the petition.

Please see attached MSDS sheets from manufacturers of calcium acetate (Attachment F).

12. Research information about the substance which includes comprehensive substance research reviews and research bibliographies including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from he National List. For petitions to include non-organic agricultural substances onto the National List. This information item should include research concerning why the substance should be permitted in the production or handling of an organic product, including the availability of organic alternatives. Commercial availability does not depend upon geographic

location or local market conditions. If research information does not exist for the petitioned substance, the petitioner should state so in the petition.

Most of the published research on calcium acetate relates to safety, effectiveness and dosage in its use in treating renal failure, which does not seem germane here. There is also some research into whether calcium acetate could be made into a less expensive and more environmentally friendly road deicer.

Many commercially available calcium products used in crop production do include calcium acetate. We have found no commercial agricultural calcium products which include only calcium acetate and only one research articles regarding the effects of calcium acetate on plants. See Attachment G for copy of this study.

13. A "Petition Justification Statement" which provides justification for any of the following actions requested in the petition. A. Inclusion of a Synthetic on the National List, §§ 205.601, 205.603,205.605(b)
• Explain why the synthetic substance is necessary for the production or handling of an organic product.
• Describe any non-synthetic substances, synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substance.
• Describe the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural methods.

Calcium acetate is taken up by plants much more rapidly than other forms of calcium. See Attachment D for a research study demonstrating the speed of uptake. Our own findings demonstrate similar results. A trial of our product by a grower in California showed a change in the color of lettuce within a day. A trial by a sod producer also showed changes within a day. Spraying hydrangeas with our product as a demonstration of pH adjustment resulted in a change in the color of the flowers overnight.

The inclusion of calcium acetate in a calcium product creates a superior product that gives plants an immediate boost of calcium and an extended release effect from the other forms of calcium included. Calcium, in addition to being necessary for cell wall formation, also aids in the uptake of other micronutrients, producing healthier plants. Plants with strong cell walls product fruits and vegetable with a longer shelf life and a greater resistance to pests. There does not appear to be an alternative to calcium acetate with regards to this immediate update property.

Calcium products such as gypsum, limestone, dolomite lime, and ground oyster shell flower can take up to 6 months to become available for uptake by crops. Gypsum and oyster shell flour frequently contain traces of heavy metals.

Calcium chloride must be applied in very small amounts when used as a foliar spray to avoid burning a crop. Calcium acetate does not have this issue.

Limestone, dolomite lime, gypsum and oyster shell flour are very heavy and are applied in bulk. Heavily laden trucks rut fields and heavily compact soil under good conditions and cannot be used under very wet conditions. Limestone, dolomite lime, gypsum, and oyster shell flour are applied in powered form and are subject to drifting. They present an inhalation hazard for the workers applying them. Limestone and dolomite lime may contain silica. All are costly to transport and some (oyster shell flour) are limited in availability.

Shade cloth is expensive and requires considerable labor to install and uninstall and is subject to wind damage. It is impractical for large operations. Application of Full Measure's sunscald prevention product is semi-automated and can be done very quickly as weather changes. National mineral/clay products are also easy to apply but have the disadvantage of being difficult to clean off produce. A large distributor of watermelons in Texas has specified the Full Measure product and indicated it will refuse produce treated with a mineral/clay product in the future after they experienced damage to pumps in their machinery used to clean melons. Pruning to prevent sunscald is also very labor intensive. Farm labor is currently in very short supply and this is likely to worsen. Pruning to prevent sunscald is not possible with some crops, may result in lower production, and is not practical for large operations. In addition, fruit may grow out from under the canopy.

Using a calcium product as a sunscald prevention has the additional advantage of providing an essential nutrient to the plant and the surrounding soil.

Growing under black plastic is a very common practice and helps cuts weeding and labor costs. In the spring, the resulting warming of soil is beneficial for plant growth, but for later crops this can be very problematic. We can document up to a 20 degree reduction in soil temperature with the use of our product. Any runoff is beneficial to the surrounding soil.

In summary, inclusion of calcium acetate on the National List would provide organic farmers with calcium products with the advantage of immediate uptake of calcium, resulting in healthier plants and produce with a longer shelf life. It would also make available an easily applied, easily removed option for preventing sunscald and for lowering the temperature beneath black plastic.

14. A Confidential Business Information Statement which describes the specific required information contained in the petition that is considered to be Confidential Business Information (CBI) or confidential commercial information and the basis for that determination.

Full Measure Industries, LLC products are patented and trademarked. Based on our patent, we consider the list of ingredients in our products to be confidential business information.

Science™

Full Measure CAL™

Liquid Calcium Acetate & Carbonate plus Humate

GUARANTEED ANALYSIS

Calcium (Ca)..30%
Derived from: Calcium Acetate, Calcium Carbonate

Also contains NON-PLANT FOOD Ingredient: Humate (Humic Acid)..1%

Patent # US7695541B1

SHAKE WELL BEFORE USE

KEEP OUT OF REACH OF CHILDREN

Manufactured For

SCIENCE PRODUCTS DIVISION
P.O. Box 333, Hanover, PA 17331

Guaranteed By

MILLER CHEMICAL & FERTILIZER, LLC
P.O. Box 333, Hanover, PA 17331

Net Weight per gallon 10.6 lb
Contents: 2x2.5 gallon (53 lb net weight) ☐

Tote 275 gallon (2,940 lb net weight) ☐

GENERAL DIRECTIONS

Full Measure CAL provides a high calcium load that combines immediately available calcium with a longer, gradual release. **Full Measure CAL** may be used on all growing crops. Soil applications should be made at 1 to 3 gallons per acre early in season or as needed by crop for best results.

FRUIT AND VEGETABLE CROPS

DRIP IRRIGATION: Use **Full Measure CAL** at the rate of 1-2 pints per acre through the drip line on a 5 to 7 day schedule or as needed.

FOLIAR APPLIED: Use **Full Measure CAL** at the rate of 1-2 pints per acre in a minimum of 20 gallons of water applied every 7 to 10 days.

SOIL APPLIED: Use **Full Measure CAL** at the rate of 1-3 gallons per acre mixed with 10-25 gallons of water per acre applied to the soil after planting and up to four weeks before harvest.

VINE AND TREE CROPS

SOIL APPLIED: Use **Full Measure CAL** at the rate of 1-3 gallons in 10-25 gallons of water applied to the soil within the drip line to provide needed calcium to the roots. Apply after petal drop and up to four weeks before harvest.

PEANUTS AND POTATOES

SOIL APPLIED: Use **Full Measure CAL** at the rate of 1-3 gallons per acre mixed with 10-25 gallons of water per acre applied to the soil before or after planting and up to 50% ground coverage of foliage. It may also be applied after first hilling on potatoes. **Spray Full Measure CAL** on hill and allow irrigation or rainfall to incorporate.

SOD FARMS AND PASTURE

SOIL APPLIED: Use **Full Measure CAL** at the rate of 1-3 gallons per acre mixed with 10-25 gallons of water per acre applied to the soil after planting and up to four weeks before harvest.

MIXING INSTRUCTIONS: Full Measure CAL is highly viscous (thick) and mixes well when combined with water and agitation. Use a minimum of 10 gallons of water per acre broadcast. When mixing **Full Measure CAL** it is best to add all other products to the applied plus water and a good anti-foaming agent to the tank first. Leave enough room for **Full Measure CAL** last in the tank. Insure that proper agitation is on and running before adding **Full Measure CAL. Full Measure CAL** is compatible with most pesticides and fungicides but a sample jar test should be performed prior to mixing to insure compatibility.

HANDLING INSTRUCTIONS FOR TOTES: Use transfer pump to move **Full Measure CAL** from tote to spray tank. 275 gallon totes are fitted with 2" male "cam lock" quick couple fitting. A female "cam lock" quick couple fitting and cut off valve on the hose will be needed.

WARNING: ***Do not pump Full Measure CAL through a filter, only lightly till into soil if necessary***

STORAGE AND DISPOSAL: Store product in the original container in a warm, dry place. Do not open product containers in fields and leave them for extended periods of time. The product will be reactive to heat. Avoid product freezing, warranty will be voided. Do not refill or re-use empty container. Recycle empty container after use in accordance with federal, state, and local regulations.

Information regarding the contents and levels of metals in this product is available on the internet at http://www.aapfco.org/metals.htm

Use this product in accordance with good agronomic practices, which include utilizing proven spray equipment set for proper coverage. Do not make applications when temperatures are too hot, as crop damage may occur. Applications should be made at temperature levels and when other environmental conditions in your area are such that your experience indicates the application will be compatible and will accomplish the desired result.

The use of this material being beyond our control and involving elements of risk to human beings, animals and vegetation, we do not make any warranty, express or implied, as to the effects of such use, when this product is not used in accordance with the direction as stated on this label.

Information concerning the raw materials composing this product can be obtained by calling 800-233-2040.

08-2015

Science™

REFLECTIONS™

Liquid Calcium Acetate & Carbonate

Patent # US7695541B1
F000501

GUARANTEED ANALYSIS

Calcium (Ca)..30%
Derived from: Calcium Acetate, Calcium Carbonate

NUTRITIONAL USE

REFLECTIONS provides a high calcium load that combines immediately available calcium with a longer, gradual release. **REFLECTIONS** may be used on all growing crops. Soil applications should be made at 1 to 3 gallons per acre early in the season for best results.

FRUIT AND VEGETABLE CROPS

DRIP IRRIGATION: Use **REFLECTIONS** at the rate of 1-2 pints per acre through the drip line on a 5 to 7 day schedule or as needed.
FOLIAR APPLIED: Use **REFLECTIONS** at the rate of 1-2 pints per acre in a minimum of 20 gallons of water applied every 7 to 10 days.
SOIL APPLIED: Use **REFLECTIONS** at the rate of 1-3 gallons per acre mixed with 10-25 gallons of water per acre applied to the soil after planting and up to four weeks before harvest.

SHAKE WELL BEFORE USE

KEEP OUT OF REACH OF CHILDREN
Manufactured For

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Net Weight per gallon 10.6 lb
Contents: 2x2.5 gallon (53 lb net weight) ☐

Tote 275 gallon (2,940 lb net weight) ☐

VINE AND TREE CROPS

SOIL APPLIED: Use **REFLECTIONS** at the rate of 1-3 gallons in 10-25 gallons of water applied to the soil within the drip line to provide needed calcium to the roots. Apply after petal drop and up to four weeks before harvest.

PEANUTS AND POTATOES

SOIL APPLIED: Use **REFLECTIONS** at the rate of 1-3 gallons per acre mixed with 10-25 gallons of water per acre applied to the soil before or after planting and up to 50% ground coverage of foliage. It may also be applied after first hilling on potatoes. **Spray REFLECTIONS** on hill and allow irrigation or rainfall to incorporate.

HEAT STRESS REDUCTION

REFLECTIONS small micron sized deposits provide excellent coverage on leaf and fruit surfaces. Deposits not immediately absorbed help regulate plant temperatures and ultra violet radiation from the sun. Proper nutritional and heat stress reduction requires applications be made prior to high heat or solar stress conditions. Multiple applications may be required to achieve and maintain adequate results. **REFLECTIONS** is not cure for sun scald. **REFLECTIONS** may also be used on black plastic mulch to reduce temperatures.

DIRECTIONS FOR USE

Apply 1 to 2 gallons of **REFLECTIONS** in 5 to 20 gallons per acre for row and vegetable crops and 50 to 100 gallons of water for tree crops.

Black Plastic Mulch - **REFLECTIONS** should be applied at 1 to 2 gallons per acre in sufficient water for coverage. A sticker such as Nu Film® 17 should be applied to allow prolonged adhesion and heat stress reduction on the plastic mulch.

MIXING DIRECTIONS

REFLECTIONS is compatible with most insecticides and fungicides. Jar test ALL materials to be used with **REFLECTIONS** prior to mixing. Add water to mixing tank and begin agitation THEN add **REFLECTIONS.**

STORAGE AND DISPOSAL: Store product in the original container in a warm, dry place. Do not open product containers in fields and leave them for extended periods of time. The product will be reactive to heat. Avoid product freezing, warranty will be voided. Do not refill or re-use empty container. Recycle empty container after use in accordance with federal, state, and local regulations.

Information regarding the contents and levels of metals in this product is available on the internet at http://www.aapfco.org/metals.htm

Use this product in accordance with good agronomic practices, which include utilizing proven spray equipment set for proper coverage. Do not make applications when temperatures are too hot, as crop damage may occur. Applications should be made at temperature levels and when other environmental conditions in your area are such that your experience indicates the application will be compatible and will accomplish the desired result.

The use of this material being beyond our control and involving elements of risk to human beings, animals and vegetation, we do not make any warranty, express or implied, as to the effects of such use, when this product is not used in accordance with the direction as stated on this label.

Information concerning the raw materials composing this product can be obtained by calling 800-233-2040.

10-2015

Attachment A

Calcium Acetate (011470) Fact Sheet

OPP CHEMICAL CODE: 011470 (CAS # 62-54-4)

Summary

Calcium Acetate is registered as a biochemical pesticide. It is a naturally occurring compound that takes form as a white odorless crystalline powder. Calcium acetate is sold as a commodity and has many industrial, medicinal, and food additive uses. With regard to direct human exposure, it is consumed orally as a medicine to reduce phosphate levels in blood, as a stabilizer in most hard candies, and as an anti-roping agent in most commercially sold breads. As a registered biochemical pesticide, it is intended for use as an attractant for yellow jackets in traps. Agency reviews indicate that the use of this compound poses no significant risk to human health or the environment due to limited exposure and negligible hazard.

I. Description of the Active Ingredient

Calcium Acetate is a white odorless powder. It occurs naturally, but is typically produced synthetically. Calcium Acetate is sold commercially and ingested regularly as both a food additive and a medicine. As a biochemical pesticide, Calcium Acetate is an attractant for yellow jackets in traps. Its active character is thought to derive from the degradation of the compound into more redolent degradation products. These more redolent sub-compounds, including acetic acid, are purported to trigger scent receptors in yellow jackets, convincing yellow jackets that a food source is near. Yellow jackets investigate the source of the scent, only to find themselves entrapped.

II. Use Sites, Target Pests, and Application Methods

- **Use Sites:** The product is largely intended for residential uses. Commercial agricultural uses are unlikely, but not prohibited. The product may be used in traps anywhere there are yellow jackets with the following qualifications. Uses are limited to non-food applications. A perimeter of 20 feet must be kept around buildings, gardens, and agricultural crops. The perimeter is to assure that volatilized residues do not contaminate food and that people in the area of the traps are not subject to the potential hazard of increased yellow jacket traffic.

- **Target pests:** Yellow jackets.

- **Application Methods:** Calcium Acetate is contained in a water soluble pouch inside a trap. The user must cut open the trap entrance structure, add the appropriate amount of

Attachment A

water to activate the attractant, and hang the trap. The trap is for a single use and is not designed to be reused or refilled. The used trap is disposed of via trash collection.

III. Assessing Risks to Human Health

No significant human health risks are expected when the pesticide is used in accordance with the label directions. The human health assessment for the 2-Methyl-1-butanol notes the following: 1) no exposure is expected due to the active ingredient's containment in traps; 2) the active ingredient is naturally occurring and is not associated with any hazard; 3) people are regularly exposed to the active ingredient as a food additive without known incident; 4) traps are intended for non-food uses only; 5) traps are to be kept at a minimum of 20 feet from buildings and food crops; 6) the active ingredient volatilizes in very low concentrations and 7) pesticidal residues resulting from volatilization dissipate and degrade rapidly.

IV. Assessing Risks to the Environment

Information submitted to the Agency in support of waiver requests indicate that the pesticide should pose no significant risk to the environment if used in accordance with label directions. The active ingredient is intended use in yellow jacket traps; accordingly, no direct exposures are expected for non-target organisms. The active ingredient volatilizes in very low concentrations and dissipates rapidly. And because it biodegrades rapidly, it is not expected to accumulate in the environment. Even in the event of small exposure, there is no ecotoxicity associated with this biochemical. Calcium acetate is an attractant with a non-toxic mode of action. There are no toxicological endpoints associated with non-target organism exposed to the compound. Further, there are no reported incidents of ecotoxicity relative to exposure to this naturally occurring compound. Because of the lack of exposure and toxicity associated with this use of Calcium Acetate, a "No Effect" (NE) determination was issued for threatened and endangered species.

V. Regulatory Information

Calcium Acetate was registered as an active ingredient in February, 2010. There is one registered product containing Calcium Acetate as an active ingredient.

VI. Registrant Inforation

Bull Run Scientific, VBT,
7400 Beaufont Springs Drive, Suite 300
Richmond, VA 23225-5519

Attachment A

VII. Additional Contact Information:

Ombudsman, Biopesticides and Pollution Prevention Division (7511P)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

Attachment B.

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Calcium Acetate

PC Code: 011470

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

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BIOPESTICIDES REGISTRATION ACTION DOCUMENT TEAM

Office of Pesticide Programs:

Biopesticides and Pollution Prevention Division

Biochemical Pesticides Branch (BPB)

Chief
Linda A. Hollis, M.S.

Senior Biologist
Russell Jones, Ph.D

Regulatory Action Leader
Menyon Adams

Product Analyst
Jacob Moore

Product Performance
Clara Fuentes, Ph.D.

I. EXECUTIVE SUMMARY:

The biochemical active ingredient Calcium Acetate is the calcium salt of acetic acid. It is an odorless white powder. It is intended for non-food outdoor use to attract eight species of yellow jackets in traps.

EPA granted waivers for all Tier I human health toxicity data requirements due to the fact that no significant human exposure by any route is anticipated from use of the trap containing this active ingredient. EPA also waived most ecological effects data requirements. All data waiver rationales are predicted on little exposure potential. An adequate efficacy study was submitted that also supported the finding that nontarget organisms are not attracted to traps baited with the attractant, when used according to label directions..

The Agency considered human exposure to calcium acetate in light of the relevant safety factors in FQPA and FIFRA. A determination has been made that there are no unreasonable adverse effects to the U.S. population in general, and to infants and children. No significant exposure via drinking water is expected from the use of the yellow jacket attractant.

Based on the information discussed above, the Agency has determined that the registered use of calcium acetate as an active ingredient will have **No Adverse Effects (NAE)** on threatened and/or endangered species. Exposure to endangered or threatened species is not expected since this is an attractant for yellow jackets and does not attract other nontarget insect species.

On October 1, 2009, EPA announced a new policy to provide a more meaningful opportunity for the public to participate on major registration decisions before they occur. According to this new policy, EPA intends to provide a public comment period prior to making a registration decision for, at minimum, the following types of applications: new active ingredients, first food use, first outdoor use, and first residential use.

Consistent with the new policy of making registration actions more transparent, Calcium Acetate has undergone a 30 day comment period as a "new active ingredient" whose registration would result in a "first outdoor use" and a "first residential use." During this process, no comments were received. Accordingly, the preliminary decision to register Calcium Acetate stands. The basis for the preliminary decision can be found in the risk assessment for Calcium Acetate, which is characterized in this BRAD. As discussed above, acute toxicity data for demonstrate that it is toxicity category IV. Calcium Acetate does not demonstrate subchronic or developmental toxicity, and it is not mutagenic or genotoxic. EPA has no concerns for any non-target organisms exposed to Calcium Acetate in accordance with approved label directions. EPA has not identified any toxic endpoints for non-target mammals, birds, plants, aquatic, or soil organisms. Nor or there concerns for any threatened and endangered species. Thus, given that Calcium Acetate has very low toxicity and presents little if any risk to non-target organisms, and efficacy data confirm its effectiveness against target pests, EPA concludes that it is in the best interests of the public and the environment to issue the registration for Calcium Acetate.

The Environmental Protection Agency (EPA) considered information submitted for granting registration under Section 3(c)(5) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and determined that the information submitted in support of Calcium Acetate yellow jacket attractant adequately satisfy current data requirements (refer to 40 CFR Subpart U § 158.2000).

II. ACTIVE INGREDIENT OVERVIEW

Common Names:	Calcium Acetate Calcium Ethanoate Acetate of lime
Chemical Names:	Calcium Acetate
Trade & Other Names:	Calcium Acetate
CAS Registry Number:	62-54-4
OPP Chemical Code:	011470
Type of Pesticide:	Attractant

Application rates and methods vary depend ing on the product. For specific information regarding the product*(s)* refer to Appendix B.

III. REGULATORY BACKGROUND

On June 16, 2007, the Agency received an application filed by Bull Run Scientific, VBT, 7400 Beaufont Springs Drive, Suite 300, Richmond, Virginia 23225-5519. Bull Run retained Cynthia Smith of Conn & Smith as their agent. Bull Run wishes to register the product Disposable Bull Run Yellow jacket Trap E containing the new biochemical active ingredient Calcium Acetate at 19.8%. A notice of receipt of this application was published in the Federal Register March 16, 2009 (74 FR 49). No comments were received following the publication of this notice. In addition, on December 15, 2009, EPA provided the opportunity for a 30-day comment period on the Agency's draft risk assessment and intention to register this pesticide product. EPA has not received any comment on this proposed action.

A. Classification

The Biochemical Classification Committee determined that Calcium Acetate is a biochemical pesticide due to its apparent non-toxic mode of action and natural occurrence in the environment.

B. Food Clearances/Tolerances

Currently, this active ingredient is not registered for use on food or feed commodities because applications are for non-food outdoor use. Therefore a tolerance or exemption from the requirement of a tolerance is not required.

IV. RISK ASSESSMENT

A. Active Ingredient Characterization

The new active ingredient calcium acetate will be formulated as an EP for use as an attractant for yellow jackets. The technical grade active ingredient (TGAI) is a white odorless powder.

Calcium acetate occurs naturally as the calcium salt of acetic acid, and has a non-toxic mode of action.

The descriptions of the product formulation and production process as well as the formation of impurities were examined by BPPD and found to be acceptable in meeting current guideline standards.

All product chemistry data requirements for registration of the new active ingredient Calcium Acetate have been satisfied.

Guideline Reference No./Property		Description of Result
830.6302	Color	White
830.6303	Physical State	Powder
830.6304	Odor	Odorless
830.6313	Stability	Stable **Not addressed for metals and metal ions**
830.6314	Oxidation/Reduction: Chemical Incompatibility	Not required for TGAI
830.6315	Flammability	Autoignition temperature = 680-730°C
830.6316	Explodability	Not required for TGAI
830.6317	Storage Stability	Not required for TGAI
830.6319	Miscibility	Not required for TGAI
830.6320	Corrosion Characteristics	Not required for TGAI
830.6321	Dielectric Breakdown Voltage	Not required for TGAI
830.7000	pH	7.2 ± 0.1 at 21°Cb
830.7100	Viscosity	Not applicable, the ingredient is a solid
830.7200	Melting Range	Decomposes to acetone at 160°C
830.7220	Boiling Range	Not applicable, the ingredient is a solid
830.7300	Density/Relative Density/Bulk Density	Approximately 30 lb/ft^3
830.7370	Dissociation Constant in Water	pKa = 4.76 at 25°C
830.7550	Partition Coefficient	Log Kow = -1.3774
830.7840	Water Solubility	400 g/L
830.7950	Vapor Pressure	0.00548 mm Hg

B. Human Health Assessment

1. Toxicology

No significant human exposure by any route is anticipated from use of this active ingredient because it is confined to a water soluble pouch inside the trap via the securely attached entrance structure. Therefore, based upon the relevant data and information, we conclude that the active ingredient is not likely to result in adverse human health effects. With regard to the Human Health Toxicity profile for Calcium Acetate, all toxicity data requirements have been waived by EPA.

a. Acute Toxicity

Data waiver rationales were granted by BPPD for all Tier I data requirements. This includes the Acute Oral Toxicity, Acute Dermal Toxicity, Acute Inhalation Toxicity, Acute Eye Irritation, Acute Dermal Irritation, Dermal Sensitization, Prenatal Developmental Toxicity, Bacterial Reverse Mutation Test, and the In Vitro Mammalian Cell Gene Mutagenicity. No additional toxicity data are required to support the nonfood use registration of this active ingredient.

b. Subchronic Toxicity

No subchronic toxicity data were submitted with this application for registration of this new active ingredient . No repeated human oral exposure is anticipated.

c. Developmental Toxicity and Mutaenicity

No developmental toxicity data were submitted with this application for registration because of little to no exposure, low toxicity, and the use pattern as a non-food use. In addition, Calcium Acetate is a naturally-occurring substance that is also approved for food-use by FDA under 40 CFR 184.1185. Also, The Agency considered human exposure to calcium acetate in light of the relevant safety factors in FIFRA. It is not expected that use of the product would result in significant human exposure when the product is used as directed. No unreasonable adverse effects are expected from exposure to this active ingredient when the product is used according to label instructions.

d. Chronic exposure and oncogenicity assessment

No chronic exposure and oncogenicity data were submitted with this application for registration because these data are not required.

e. Effects on the Endocrine System

EPA is in the process of issuing test orders for endocrine effects. The schedule for issuance of test orders, and details regarding status is available at http://www.epa.gov/endo/. EPA has also established a docket for the test orders in www.regulations.gov under docket number EPA-HQ-OPP-2009-0634.

Data required under the test orders will provide information to help EPA identify whether chemicals have the potential to interact with the estrogen, androgen, and/or thyroid hormone systems, which regulate growth, metabolism, development, and reproduction. The data generated from the screens will provide robust and systematic scientific information that will help EPA identify whether additional testing is necessary.

Calcium Acetate is a naturally occurring substance. To date, there is no evidence to suggest that our natural exposure to Calcium Acetate affects the immune system, functions in a manner similar to any known hormone, or that it acts as an endocrine disruptor. Moreover, the use of Calcium Acetate is not expected to result in any significant exposures, effectively obviating any opportunity for negative effects on humans or the environment. Therefore, it is unlikely that Calcium Acetate will have estrogenic or endocrine effects.

2. Dose Response Assessment

A dose response assessment was not conducted because based on the proposed use of the product, human exposure is not expected.

3. Dietary Exposure and Risk Characterization

The active ingredient Calcium Acetate is intended for non-food uses. Accordingly, it is not expected to pose any direct dietary risk to humans.

With regard to any incidental exposure, all routes of exposure associated with the active ingredient's use as an attractant are negligible. Additionally, the active ingredient occurs naturally in fruit and is a well-recognized flavor agent in food additives. Its regular consumption in a human diet is not associated with any hazards. Finally, the acute toxicity information on file indicates that the risks associated with even incidental exposures would be negligible.

4. Drinking Water Exposure Risk Characterization

No significant drinking water exposure is expected from Calcium Acetate because of its contained in a water soluble pouch inside the trap.

5. Acute and Chronic Dietary Exposure and Risks for Sensitive Subpopulations, Particularly Infants and Children

Based on the non-food use pattern, the limited potential for even incidental exposure, and the dietary toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result to the United States population, including infants and children, from aggregate exposure to any incidental residues of Calcium Acetate.

6. Occupational, Residential, School and Day Care Exposure and Risk Characterization

a. Occupational Exposure and Risk Characterization

There is little likelihood of occupational exposure via oral, dermal, or ocular routes due to the fact that the end product is in a trap. Inhalation exposure is minimal and not likely to occur at levels that would be toxic due to the low concentration of Calcium Acetate in the end-use product (19.8%).

b. Residential, School and Day Care Exposure and Risk Characterization

No indoor residential uses are currently approved. No exposure is anticipated to children at school or day care facilities because the pesticide is enclosed in a water soluble pouch inside a trap.

7. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

The potential for aggregate exposure is expected to be insignificant. Calcium Acetate is not expected to be present in quantities greater than 2.466×10^9 g/liter air/week, and limited only to those times when yellow jackets, hornets and wasps are active. Given a lack of acute toxicological endpoints for Calcium Acetate, the aggregate exposure scenario presents no significant concerns for risk.

8. Cumulative Effects

Calcium Acetate is intended for non-food uses as an attractant in a trap. No dietary exposures are expected. To the degree that there might be any incidental dietary exposure, Calcium Acetate does not share any known common mechanism of toxicity with other substances.

9. Risk Characterization

The Agency considered human exposure to Calcium Acetate in light of the relevant safety factors in FQPA and FIFRA. A determination has been made that no unreasonable adverse effects to the U.S. population in general, and to infants and children in particular, will result from the use of the calcium acetate insecticide

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Hazards (Relative to the Biochemical Pesticides Nontarget Organisms and Environmental Fate Data Requirements- 40 CFR§158.2060)

Calcium Acetate is contained in a water soluble pouch in a trap. The user must cut open the trap entrance structure, add the appropriate amount of water to activate the attractant, and hang the trap. The trap is for a single use and is not designed to be reused or refilled. The used trap is disposed via trash collection. Efficacy data were submitted that demonstrated that nontarget organisms are not attracted to the Calcium Acetate trap. (MRID 47255003)

2. Environmental Fate and Ground Water Data

The need for environmental fate and groundwater data was not triggered because results of the acute toxicity assessment did not trigger any additional Tier I studies.

3. Ecological Exposure and Risk Characterization

The active ingredient is intended for formulation of end use products that are placed in insect traps for residential or agricultural use. When used according to the proposed label directions, no direct exposures are expected for nontarget organisms. Moreover, the active ingredient is used at a low concentration as an attractant, and is not expected to accumulate in the environment. Given these characteristics of Calcium Acetate, nontarget exposure and ecological effects studies were waived.

4. Threatened and Endangered Species Assessment

Based on the available data, a No Effects (NE) determination was made Calcium Acetate on threatened and endangered species when the product is used according to label use directions. Specifically, in MRID 47451404, efficacy testing showed no honeybees or other non-target insect species were attracted or caught by the Calcium Acetate product. Since the active ingredient in contained in a trap, there is no exposure to birds, fish, aquatic invertebrates, or other non-target organisms.

D. PRODUCT PERFORMANCE DATA (EFFICACY)

Submission of product performance data (OPPTS 810.3000) is listed as a requirement for all pesticide products. Customarily, the Agency requires efficacy data to be submitted for review only in connection with the registration of products directly pertaining to the mitigation of disease bearing human health organisms and certain designated quarantine pests, i.e., ticks, mosquitoes, fleas, Mediterranean fruit flies, gypsy moths, Japanese beetles, etc. For a list of organisms considered by the Agency as "public health pests", please refer to Pesticide Registration Notice 2002-1 (http://www.epa.gov/PR_Notices/pr2002-1.pdf).

A report on the efficacy of the product was submitted because the yellow jacket wasp is a public health pest. The study consisted of 7 field trials conducted on different dates and at different locations representing different habitats. Traps were emptied and rebaited periodically. Traps were rotated independently of replenishment, to avoid possible positional effects. Insects were collected for identification. Data was analyzed on mean number of insects per trap per day. Treatment means were compared to control means using a Paired t-Test with P set 0.05 significance level. Replicates with no recorded catch were omitted from the analyses. Data was summarized by average species caught per trap per treatment, and by average species per trap across all the treatment pooled together. The study results showed statistically significant difference between treatment and control traps in number of catches for all wasps.

V. Risk Management Decision

A. Determination of Eligibility for Registration

Section 3(c)(5) of FIFRA provides for the registration of a new active ingredient if it is determined that (A) its composition is such as to warrant the proposed claims for it; (B) its labeling and other materials required to be submitted comply with the requirements of FIFRA; (C) it will perform its intended function without unreasonable adverse effects on the environment; and (D) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

The four criteria of the Eligibility Determination for Pesticidal Active Ingredients are satisfied by the science assessments supporting the product containing Calcium Acetate. This product is not expected to cause unreasonable adverse effects and is likely to act as a yellow jacket attractant when used according to label instructions. Therefore, EPA concludes that Calcium Acetate is eligible for registration for the labeled uses.

B. Regulatory Decision

On October 1, 2009, EPA announced a new policy to provide a more meaningful opportunity for the public to comment on major registration decisions before they occur. According to this new policy, EPA intends to provide a public comment period prior to making a registration decision for, at minimum, the following types of applications: new active ingredients; first food use; first outdoor use; and first residential use. Accordingly, this pesticide was subject to a 30-day comment period as a new active ingredient with both outdoor uses and residential uses. No comments were received during that comment period.

At this time, EPA believes, the data submitted fulfill the requirements of registration for products containing Calcium Acetate for use to attract yellow jackets, wasps and hornets. Acute toxicity data for Calcium Acetate demonstrate that it is toxicity category IV for all routes of exposure. Calcium Acetate does not demonstrate subchronic or developmental toxicity, and it is not mutagenic or genotoxic. EPA has no concerns for any non-target organisms exposed to Calcium Acetate in accordance with approved label directions. EPA has not identified any toxic endpoints for non-target mammals, birds, plants, aquatic, or soil organisms. Nor are there concerns for any threatened and endangered species. Given the non-toxic character of Calcium Acetate, EPA supports its registration under Section 3(c) (5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Refer to Appendix B for product-specific information.

1. Conditional/Unconditional Registration

All data requirements are fulfilled and EPA has determined that an unconditional registration for Calcium Acetate is warranted under Section 3(C) (5) of FIFRA.

C. Labeling

Before releasing pesticide products containing Calcium Acetate for shipment, the applicant is required to provide appropriate labels.

D. Environmental Justice

EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical, unusually high exposure to calcium acetate compared to the general population. Please comment if you are aware of any sub-populations that may have atypical, unusually high exposure compared to the general population.

VI. ACTIONS REQUIRED BY REGISTRANTS

The Agency evaluated all of the data submitted in connection with the initial registration of Calcium Acetate and determined that these data are sufficient to satisfy current registration data requirements. No additional data are required to be submitted to the Agency at this time.

Notwithstanding the information stated in the previous paragraph, it should be clearly understood that certain specific data are required to be reported to the Agency as a requirement for maintaining the Federal registration for a pesticide product. A brief summary of these types of data are listed below.

A. Reporting of Adverse Effects

Reports of all incidents of adverse effects to the environment must be submitted to the Agency under the provisions stated in FIFRA, Section 6(a)(2).

B. Reporting of Hypersensitivity Incidents

Additionally, all incidents of hypersensitivity (including both suspected and confirmed incidents) must be reported to the Agency under the provisions of 40 CFR Part 158.2050(d).

VII. Appendix A. Data Requirements (40 CFR Part 158-Subpart U)

*NOTE: MRID numbers listed in the following tables are representative of supporting data for the original registration of the product containing this active ingredient. Subsequent to this registration, there may be additional MRIDs that support registration of other products containing this active ingredient.

Table 2. Human Toxicology Data Requirements for Calcium Acetate (40 CFR § 158.2050)

Study Type, Species, OPPTS Guideline	Regulatory Decision	LD_{50}/LC_{50}/LOAEL/NOAEL Results	Toxicity Category	MRID Review Date
Acute Oral Toxicity, rat, OPPTS 870.1100	Acceptable Waiver	N/A	WAIVED	47451405

Acute Dermal Toxicity, rat, OPPTS 870.1200	Acceptable Waiver	N/A	WAIVED	47451405
Acute Inhalation Toxicity, rat, OPPTS 870.1300	Acceptable Waiver	N/A	WAIVED	47451405
Acute Eye Irritation, rabbit, OPPTS 870.2400	Acceptable Waiver	N/A	WAIVED	47451405
Acute Dermal Irritation, rabbit, OPPTS 870.2500	Acceptable Waiver	N/A	WAIVED	47451405
Dermal Sensitization OPPTS 870.2600	Acceptable Waiver	N/A	WAIVED	47451405
Prenatal Development OPPTS 870.3700	Acceptable Waiver	N/A	WAIVED	47451405
Reproductive Toxicity OPPTS 870.3800	Acceptable Waiver	N/A	WAIVED	47451405

Table 2. Human Toxicology Data Requirements for Calcium Acetate (40 CFR § 158.2050)

Study Type, Species, OPPTS Guideline	Regulatory Decision	LD_{50}/LC_{50}/LOAEL/NOAEL Results	Toxicity Category	MRID Review Date
Bacterial Reverse Mutation Test OPPTS 870.5100	Acceptable Waiver	N/A	WAIVED	47451405
In vitro Mammalian Cell Gene Mutation Assay OPPTS 870.5300	Acceptable Waiver	N/A	WAIVED	47451405
Acute Neurotoxicity OPPTS 870.6200	Acceptable Waiver	N/A	WAIVED	47451405

Table 3. Nontarget Organism, Fate and Expression Data Requirements for Calcium Acetate (40 CFR § 158.2060)		
Study/OPPTS Guideline No.	**Results**	**MRID #(s)**
Avian acute oral toxicity (850.2100)	Waived	47451404
Vegetative Vigor (850.4150)	Waived	47451404
Nontarget insect toxicity (Honey bee) (850.3020)	Waived	47451404
Aquatic Invertebrate Acute Toxicity (850.1010)	Waived	47451404
Seeding Emergence (850.4100)	Waived	47451404
Oyster Acute Toxicity (850.1025)	Waived	47451404
Fish Acute Toxicity, Freshwater (850.1075)	Waived	47451404
Daphnid Chronic Toxicity (850.1300)	Waived	47451404
Fish Early-Life Stage Toxicity (850.1400)	Waived	47451404
Avian Dietary Toxicity (850.2200)	Waived	47451404
Avian Reproduction (850.2300)	Waived	47451404
Aquatic Plant Toxicity (850.4400)	Waived	47451404
Algal Toxicity (850.5400)	Waived	47451404
Nontarget insect testing (850.4350)	Waived	47451404

VIII. GLOSSARY OF ACRONYMS AND ABBREVIATIONS

BPPD	Biopesticides and Pollution Prevention Division
BRAD	Biopesticide Registration Action Document
CFR	Code of Federal Regulations
cm^3	cubic centimeter
CSF	Confidential Statement of Formula
°C	degrees Celsius
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EPA	Environmental Protection Agency (the "Agency")
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
FR	Federal Register
g	gram
kg	kilogram
L	liter

LD_{50}	median lethal dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, or inhalation). It is expressed as a weight of substance per unit weight of animal (e.g., mg/kg).
MRID No.	Master Record Identification Number
mg	milligram
mL	milliliter
MP	manufacturing-use product
MPCA	microbial pest control agent
NE	"No Effect"
NIOSH	National Institute for Occupational Safety and Health
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
PCR	polymerase chain reaction
PPE	personal protective equipment
TGAI	technical grade of the active ingredient

IX. Appendix B.

For product specific information, please refer to http://www.epa.gov/pesticides/pestlabels

X. Appendix C.

REFERENCES

MRID 47451401. Smith, C. June 13, 2008. Calcium Acetate: Product Identity, Composition and Analysis.

MRID 47451402. Smith, C. June 13, 2008. Calcium Acetate: Color, Physical, State, Odor, Stability, UV/Visible Lights Absorption, Melting Point, Density, Dissociation Constant, Partition Coefficient, Water Solubility and Vapor

MRID 47451403. Smith, C. June 13, 2008. Calcium Acetate: pH.

MRID 47451404. Smith, C. June 13, 2008. Calcium Acetate (TGAI) & Disposable Bull Run Yellow jacket Trap E. Environmental Fate & Effects on Non target organisms.

MRID 47451405. Smith, C. June 13, 2008. Calcium Acetate (TGAI) Mammalian Toxicology.

Attachment C

4 Chemical and Physical Properties

4.1 Computed Properties

Property Name	Property Value
Molecular Weight	158.166 g/mol
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	4
Rotatable Bond Count	0
Complexity	25.5
CACTVS Substructure Key Fingerprint	AAADcYBgOAAAAgAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGgAAAAAAAACAgAACCAAAAAAIAACQCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Topological Polar Surface Area	80.3 A^2
Monoisotopic Mass	157.989 g/mol
Exact Mass	157.989 g/mol
Compound Is Canonicalized	true
Formal Charge	0
Heavy Atom Count	9
Defined Atom Stereocenter Count	0
Undefined Atom Stereocenter Count	0
Defined Bond Stereocenter Count	0
Undefined Bond Stereocenter Count	0
Isotope Atom Count	0
Covalently-Bonded Unit Count	3

▸ *from PubChem*

4.2 Experimental Properties

4.2.1 Physical Description

1. DryPowder
2. Liquid

3. OtherSolid
4. PelletsLargeCrystals

▸ *from EPA Chemicals under the TSCA*

Anhydrous calcium acetate is a white, hygroscopic, bulky, crystalline solid with a slightly bitter taste. A slight odour of acetic acid may be present. The monohydrate may be needles, granules or powder

▸ *from EU Food Improvement Agents*

WHITE-TO-BROWN OR GREY CRYSTALS WITH CHARACTERISTIC ODOUR.

▸ *from ILO-ICSC*

4.2.2 Color

COLORLESS CRYSTALS

Weast, R.C. (ed.). Handbook of Chemistry and Physics. 57th ed. Cleveland: CRC Press Inc., 1976., p. B-98

▸ *from HSDB*

Rod-shaped crystals

Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 270

▸ *from HSDB*

White, hydrogroscopic, crystalline solid

Ashford, R.D. Ashford's Dictionary of Industrial Chemicals. London, England: Wavelength Publications Ltd., 1994., p. 166

▸ *from HSDB*

4.2.3 Odor

SLIGHT ODOR OF ACETIC ACID

Sax, N.I. Dangerous Properties of Industrial Materials. 4th ed. New York: Van Nostrand Reinhold, 1975., p. 507

▸ *from HSDB*

4.2.4 Melting Point

> 160 °C

▸ *from DrugBank*

4.2.5 Solubility

37.4 G IN 100 CC OF WATER @ 0 DEG C; 29.7 G IN 100 CC OF WATER @ 100 DEG C; SLIGHTLY SOL IN ALCOHOL

Weast, R.C. (ed.). Handbook of Chemistry and Physics. 57th ed. Cleveland: CRC Press Inc., 1976., p. B-98

▸ *from HSDB*

Practically insol in acetone and benzene

Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 270

▸ *from HSDB*

in water: very good

▸ *from ILO-ICSC*

4.2.6 Density

1.50 kg/l

Ashford, R.D. Ashford's Dictionary of Industrial Chemicals. London, England: Wavelength Publications Ltd., 1994., p. 166

▸ *from HSDB*

1.5 g/cm^3

▸ *from ILO-ICSC*

4.2.7 Stability

VERY HYGROSCOPIC ...

Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 270

▸ *from HSDB*

4.2.8 Decomposition

When heated to decomposition ... emits acrid smoke and fumes.

Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 627

▸ *from HSDB*

160°C

▸ *from ILO-ICSC*

4.2.9 pH

6,0-9,0 (10 % aqueous solution)

▸ *from EU Food Improvement Agents*

0.2 molar aq soln: 7.6 /Monohydrate form/

Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 270

▸ from HSDB

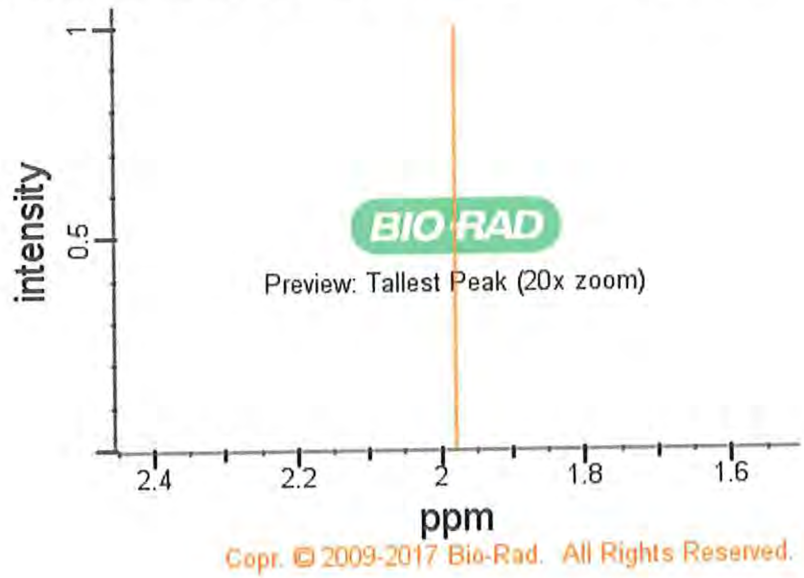
4.3 Spectral Properties

INDEX OF REFRACTION: 1.55

Weast, R.C. (ed.). Handbook of Chemistry and Physics. 57th ed. Cleveland: CRC Press Inc., 1976., p. B-98

▸ from HSDB

4.3.1 H1-NMR

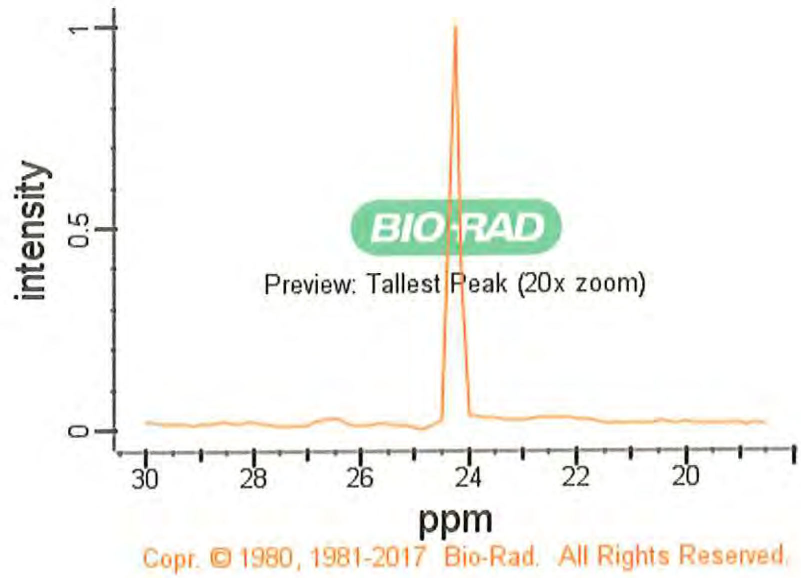
Instrument Name	Varian CFT-20
Copyright	
Thumbnail	

▸ from SpectraBase

4.3.2 C13-NMR

Source of Sample	Fluka AG, Buchs, Switzerland
Copyright	

Thumbnail



▸ *from SpectraBase*

Attachment D

We've made some changes to EPA.gov. If the information you are looking for is not here, you may be able to find it on the EPA Web Archive or the January 19, 2017 Web Snapshot.

EPA United States Environmental Protection Agency

Safer Chemical Ingredients List

EN ESPAÑOL

Related Information

For chemical manufacturers and raw material suppliers looking for information on how to list a chemical on the Safer Chemical Ingredients List (SCIL), visit our step-by-step guide.

On this page:

- Safer Chemical Ingredients List
- Overview of the Safer Chemical Ingredients List
- Technical notes about the list
- Additional resources

A downloadable spreadsheet of the Safer Chemical Ingredients List (17 pp, 401 K) is also available. (Download Excel Viewer) EXIT See the "Updates" tab in the Excel spreadsheet for recently added and/or updated chemicals.

Safer Chemical Ingredients List

- The listed chemicals are safer alternatives, grouped by their functional-use class.†

		Number	Use
○	Calcium acetate	62-54-4	Processing Aids and Additives
○	Calcium magnesium acetate	76123-46-1	Processing Aids and Additives

Showing 1 to 2 of 2 entries (filtered from 918 total entries)

Previous 1 Next

Please note: Use of chemicals from the list in product formulations does not entitle the manufacturer to make any claims related to the EPA or the Safer Choice Program or label. Manufacturers may only make such claims after going through formal third-party Safer Choice review, qualifying for the Label based on both ingredient- and product-level criteria (addressing, for example, issues of ingredient purity and physical form, potential synergistic effects, and performance), and entering an EPA Safer Choice partnership. For more information see Steps to Get the Safer Choice Label.

Overview of the Safer Chemical Ingredients List

The Safer Chemical Ingredients List (SCIL) is a list of chemical ingredients, arranged by functional-use class, that the Safer Choice Program has evaluated and determined to be safer than traditional chemical ingredients. This list is designed to help manufacturers find safer chemical alternatives that meet the criteria of the Safer Choice Program.

Before Safer Choice decides to include a chemical on the SCIL, a third-party profiler (i.e., NSF, International or ToxServices) gathers hazard information from a broad set of resources, including the identification and evaluation of all available toxicological and environmental fate data. The third party profiler submits a report to Safer Choice, with a recommendation on whether the chemical passes the Criteria for Safer Chemical Ingredients. Safer Choice staff performs due diligence by reviewing the submission for completeness, consistency, and compliance with the Safer Choice Criteria. If more than one third-party has

Green half-circle - The chemical is expected to be of low concern based on experimental and modeled data. Additional data would strengthen our confidence in the chemical's safer status.

Yellow triangle - The chemical has met Safer Choice Criteria for its functional ingredient-class, but has some hazard profile issues. Specifically, a chemical with this code is not associated with a low level of hazard concern for all human health and environmental endpoints. (See Safer Choice Criteria). While it is a best-in-class chemical and among the safest available for a particular function, the function fulfilled by the chemical should be considered an area for safer chemistry innovation.

Grey square - This chemical will not be acceptable for use in products that are candidates for the Safer Choice label and currently labeled products that contain it must reformulate per Safer Choice Compliance Schedules.

Note: Some functional class listings contain both green circle and yellow triangle chemicals because the yellow triangle chemicals fill a functional need not met by the available green circle chemicals. For example, yellow triangle preservatives are needed to meet the full range of antimicrobial efficacy, and yellow triangle solvents are needed to meet restrictions on volatile organic compounds (VOCs) in certain product classes.

Additional Resources

See the following resources for additional information on the chemicals in cleaning products:

- CleanGredients® EXIT

  This database of safer chemicals supports the Safer Choice Program by providing a list of chemicals, arranged by component class, that meet the Safer Choice Criteria.

- Consumer Specialty Products Association (CSPA) Consumer Product Ingredients Dictionary EXIT

Attachment E

The Carcinogenic Potency Project

Calcium acetate (CAS 62-54-4)

SMILES, InChI and Structure are below.

Rats and Mice: Cancer Test Summary

Rat Target Sites		Mouse Target Sites		TD_{50} (mg/kg/day)	
Male	Female	Male	Female	Rat	Mouse
no positive	no test	no test	no test	no positive	no test

Key to the Table Above

Positivity: For each chemical with a positive (carcinogenic) experiment in the Carcinogenic Potency Database (CPDB), results are included on carcinogenic potency (TD_{50}) in each species and target sites in males and females. Positivity is determined by an author's opinion in a published paper. If all experimental results in the CDPB are negative in a sex-species group, "no positive" appears. If the CPDB has no experiments in the sex-species group, "no test" appears. The summary presents the strongest evidence of carcinogenicity in each group. If there are both positive and negative experiments in a sex-species, the negative results are ignored in this Summary Table.

Target Site Codes: Target sites are listed if any author of published experimental results concluded that tumors were induced in that organ by the test agent. If there is more than one positive experiment in a sex-species, target sites listed may be from more than one experiment, e.g. if liver and lung are both listed, then liver may have been a target in one experiment and lung in another.

TD_{50}: Our standardized measure of carcinogenic potency, TD_{50}, is the daily dose rate in mg/kg body weight/day to induce tumors in half of test animals that would have remained tumor-free at zero dose. Whenever there is more than one positive experiment in a species, the reported TD_{50} value is a Harmonic Mean calculated using the TD_{50} value from the most potent target site in each positive experiment.

The Carcinogenic Potency Database (CPDB) is a unique and widely used international resource of the results of 6540 chronic, long-term animal cancer tests on 1547 chemicals. The CPDB provides easy access to the bioassay literature, with qualitative and quantitative analyses of both positive and negative experiments that have been published over the past 50 years in the general literature through 2001 and by the National Cancer Institute/National Toxicology Program through 2004. The CPDB standardizes the diverse literature of cancer bioassays that vary widely in protocol, histopathological examination and nomenclature, and in the published author's choices of what information to provide in their papers. Results are reported in the CPDB for tests in rats, mice, hamsters, dogs, and nonhuman primates.

For each experiment, information is included on species, strain, and sex of test animal; features of experimental protocol such as route of administration, duration of dosing, dose level(s) in mg/kg body weight/day, and duration of experiment; experimental results are provided on target organ, tumor type, and tumor incidence; carcinogenic potency (TD_{50}) and its statistical significance; shape of the dose-response, author's opinion as to carcinogenicity, and literature citation.

Only tests with dosing for at least ¼ the standard lifespan of the species and experiment length at least ½ the lifespan are included in the CPDB. Only routes of administration with whole body exposure are included. Doses are standardized, average dose rates in mg/kg/day. A description of methods used in the CPDB to standardize the diverse literature of animal cancer tests is presented for: 1) Criteria for inclusion of experiments 2) Standardization of average daily dose levels and 3) TD_{50} estimation for a standard lifespan. See Methods for other details.

TD_{50} provides a standardized quantitative measure that can be used for comparisons and analyses of many issues in carcinogenesis. The range of TD_{50} values across chemicals that are rodent carcinogens is more than 100 million-fold. More than half the chemicals tested are positive in at least one experiment.

A plot of all results on each experiment in the CPDB for this chemical is presented below. These results are the source information for the Cancer Test Summary table above.

Calcium acetate: All Experiments and Citations in CPDB

The definition of each code in the plot below will appear in a pop-up window when the field name in the header line is clicked, e.g., Strain, Site, Path. Each numbered line starts a new experiment and reports protocol information in black. Average daily dose-rates per kg body weight per day are in green. Remaining lines report experimental results in blue.

Abbreviations of fields in header line: # = the line number in the plot of all CPDB chemicals; Xpo = duration of dosing; Xpt = duration of experiment; Site = tissue; Path = tumor type; DR = dose-response; AuOp = author's opinion about carcinogenicity; LoConf, UpConf = confidence limits (99%) on TD_{50}; Inc = tumor incidence for each dose group.

See Guide to reading the plot for details on each field, using an example of one experiment.

See Help to improve readability, or to fit the plot onto the screen or a printed page.

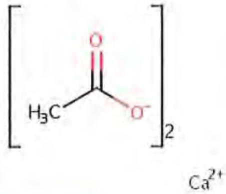
```
Chemical (Synonym) CAS
# Species Sex Strain Route Xpo+Xpt PaperNum        0 Dose  1 Dose 2 Dose  3 Dose         Literature Reference or NCI/NTP:Site Path
Site Path Notes  TD50  DR Pval   AuOp LoConf UpConf  Cntrl   1 Inc  2 Inc  3 Inc                                    Brkly Code

CALCIUM ACETATE   62-54-4
1047 R m sda eat 79w79 1709                          0     1.20gm                         Kasprzak;carc,6,279-282;1985
 kid tum e       no dre   P=1.       4.28gm n.s.s.  0/30    0/30
 liv tum e       no dre   P=1.       4.28gm n.s.s.  0/30    0/30
```

SMILES Code for Calcium acetate: CC(=O)[O-].[O-]C(=O)C.[Ca+2]

InChI Code for Calcium acetate: InChI=1/2C2H4O2.Ca/c2*1-2(3)4;/h2*1H3,(H,3,4);/q;;+2/p-2

Source for SMILES and InChI: USEPA Distributed Structure-Searchable Toxicity (DSSTox) Database

Chemical Structure for Calcium acetate:



Source for structure: National Library of Medicine ChemIDPlus

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See full CPDB Summary Table on 1547 chemicals. See Full CPDB for all results on 6540 experiments of 1547 chemicals.

A complete list of CPDB chemicals, which is searchable by name or by CAS number, is available here.

For a compendium of CPDB results organized by target organ, which lists all chemicals in each species that induced tumors in each of 35 organs, see Summary Table by Target Organ.

The CPDB is available in several formats that permit printing and downloading into spreadsheets and statistical databases.

1. A plot of the CPDB presents results of 1547 experiments on 6540 chemicals in an easily readable format that has been used in publications of the CPDB.
2. A Screen version plot for use on a single computer screen, with the same data.
3. Excel version of the same data.
4. Tab-separated versions of the same data, which can be easily read into databases.

A Supplementary Dataset gives details on dosing and survival for each experiment.

Relatively precise estimates of the lower confidence limit on the TD_{10} (LTD_{10}) are readily calculated from the TD_{50} and its lower confidence limit, which are reported in the CPDB. For researchers and regulatory agencies interested in LTD_{10} values, we provide them in an Excel spreadsheet.

PDF versions of our publications of analyses using the CPDB are available, organized by year and by research topic.

Carcinogenic Potency Database Project (CPDB) Home Page
For more information about this Web Page, contact Specialized Information Services (tehip@teh.nlm.nih.gov).
Last updated: October 3, 2007

PDF documents are best viewed with the free Adobe® Reader http://get.adobe.com/reader
Excel documents are best viewed with the free Excel® Viewer http://www.microsoft.com/en-us/download/details.aspx?id=10

Attachment F

Blaser Swisslube Inc.
31 Hatfield Lane, Goshen, New York • Tel. (845) 294-3200 • Fax (845) 294-3102

Blaser. SWISSLUBE

MSDS Calcium Acetate, Liquid

PRODUCT IDENTIFICATION

HMIS III®[1]	
Health	1
Flamability	0
Physical Hazard	0
Personal Protection	A

Manufacturer Blaser Swisslube Inc.
31 Hatfield Lane
Goshen, NY 10924

Emergency phone number USA: (845) 294-3200

Product name: **Calcium Acetate, Liquid** ***Art. 29111***

Product type: Calcium Acetate in water

Prepared by: OH & S Coordinator

Date of issue: April 23, 2007

Supersedes: Edition 4 of June 21, 2005

PRODUCT COMPOSITION

This product is a mixture of:

Ingredients:	%	CAS#
Water	Balance	7732-18-5
Calcium Acetate	<25	62-54-4
Triadine 3	<0.5	4719-04-4, 7732-18-5

EMERGENCY & FIRST AID PROCEDURES

Inhalation: If large amounts are inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen and call a physician.

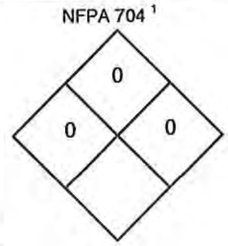
Skin contact: Wash with plenty of soap and water (good personal hygiene practices are sufficient). Remove any contaminated clothing and launder before reuse.

Eye contact: Rinse with plenty of fresh water for 15 minutes. Consult physician if necessary.

Ingestion: Give large amounts of liquid to dilute. Seek medical attention as soon as possible.

FIRE & EXPLOSION HAZARD DATA

NFPA 704[1]



Flash point: Not combustible

Explosion limits: Not applicable

Fire fighting media: Will not burn. In case of fire, use extinguishing method suitable for surrounding materials

Hazardous combustion products: Not applicable

Products formed under abnormal conditions: Thermal decomposition above 320°F (160°C) can produce acetone vapors

Unusual fire or explosive hazards: None expected

PRECAUTIONS FOR SAFE HANDLING & STORAGE

Steps to be taken in case material is leaked or spilled: Contain spill and recover free product. Absorb remainder using absorbent materials (such as 'Speedy Dry®'). Spill areas can be washed with water; collect wastewater for approved disposal. Prevent material from entering sewer.

Waste disposal methods: Dispose according to all applicable federal, state and local regulations.

Precautions to be taken in handling/storing: Wash thoroughly after handling.

Other precautions: Do not store with strong oxidizers.

[1] See last page for explanations

EXPOSURE CONTROL MEASURES & PERSONAL PROTECTION

Ventilation requirements:	Ventilation sufficient to comply with recommended NIOSH exposure limit for metalworking fluids is suggested.
Respiratory protection:	Not generally required.
Protective gloves:	Not generally required. Impervious gloves recommended where prolonged or repeated contact cannot be avoided. Use caution when wearing gloves around moving machinery.
Eye protection:	Industrial safety glasses are recommended.
Other protective equipment or clothing:	Standard work clothing and shoes.
Work/hygienic practices:	Thorough personal hygiene and clean working practices are sufficient.

CHEMICAL & PHYSICAL PROPERTIES OF THE COMPLETE PRODUCT

Volatiles in %:	Not determined
Boiling point:	Not determined
Freezing point:	Not determined
pH:	8.8
Density:	1.126 g/cm^3
Solubility in water:	Soluble
Appearance and odor:	Opaque liquid / slight vinegar smell

REACTIVITY DATA

Stability:	Stable
Incompatibilities with other materials:	Keep away from strong oxidizers.
Hazardous decomposition or by-products:	Thermal decomposition above 320°F (160°C): Acetone vapor
Hazardous polymerization or by-products:	Will not occur

HEALTH HAZARD DATA

LD_{50}	> 5 g/kg (calculated)
Health hazards (acute/chronic):	None
Skin irritation:	Not expected to be an irritant
Eye irritation:	Not expected to be an irritant
Carcinogenicity:	None of the ingredients are listed on OSHA, NTP IARC or other list of carcinogenic substances
Routes of entry:	• Inhalation: Unlikely • Skin: Not readily absorbed through skin • Ingestion: Accidental only
Signs and symptoms of exposure:	None established
Medical conditions generally aggravated by exposure:	Not established
OSHA regulated:	No
WHMIS regulated:	No
Exposure limits:	***NIOSH*** recommended exposure limit for metalworking fluids: 0.5 mg/m^3

ENVIRONMENTAL, REGULATORY AND SUPPLEMENTAL INFORMATION

NFPA Storage: III B

SARA Title III information:

- Immediate health (acute): No
- Fire hazard: No
- Sudden pressure release: No
- Reactive hazard: No
- Delayed health (chronic): No

This product does not contain any ingredients listed on the SARA Title III, Section 313 List of Chemicals.

RCRA / Hazardous Waste: This product, as sold, does not meet the criteria of a hazardous waste as defined under 40 CFR 261, in that it does not exhibit the characteristics of a hazardous waste of Subpart C, nor is it listed as a hazardous waste under Subpart D. It is the end user's responsibility to determine the regulatory status of the waste at the time of disposal and dispose of the waste according to all federal, state and local regulations.

TSCA: All ingredients of this product are listed on the TSCA Chemical Substances Inventory.

Clean Air Act: This product **does not contain nor is it manufactured** with ozone depleting substances as defined in the Federal Clean Air Act Amendments of 1990, sections 602 and 611.

Canadian DSL / NDSL information: All ingredients of this product are listed on Canadian DSL

DOT: This product is **not** considered hazardous under current **DOT** regulations.

RoHS: This product conforms to the RoHS Directive in that the RoHS regulated materials are absent or their concentrations are significantly below regulatory thresholds.

Animal products / byproducts: This product does not contain any raw materials sourced from animal products or animal byproducts.

HMIS III® Ratings	0	1	2	3	4
Health, Flamability, Physical Hazard	Minimal hazard	Slight hazard	Moderate hazard	Serious hazard	Severe hazard

There is a new generation of HMIS® being introduced, HMIS® III. This new system still uses colored bars and numeric hazard ratings, but instead of "reactivity" it uses "physical hazard" warnings, which are more in line with OSHA defined hazards. HMIS® III will provide employees the tools to understand and handle chemicals with a far greater degree of precision, thereby increasing the potential for the customer's HazCom compliance.

The HMIS® III health criteria have been designed to be as compatible as possible with the definitions contained in ANSI Z129.176. They also reflect certain criteria accepted by NIOSH, U.S. EPA, and other organizations. Acute oral, dermal, and inhalation toxicity are rated on a five-tiered scale (0-4), generally with a tenfold difference. The new HMIS® III uses two boxes to indicate health hazards:

- The left box indicates if the chemical is a chronic or an acute hazard. An asterisk (*) or other designation corresponds to additional information on data sheet or separate chronic effects notification.
- The right box is used to indicate the hazard rating number. Numeric ratings are based on acute toxicity and chronic health hazard data, either from internal company data or published information. The HMIS® III health criteria have been designed to be as compatible as possible with the definitions contained in ANSI Z129.176. They also reflect certain criteria accepted by NIOSH, U.S. EPA, and other organizations. Acute oral, dermal, and inhalation toxicity are rated on a five-tiered scale (0-4), generally with a tenfold difference between the levels.

Personal Protection Index:
A: Safety glasses with side shields

NFPA 704 Ratings (Under Fire Conditions)	0	1	2	3	4
Health Hazard	Normal Material	Slightly Hazardous	Hazardous	Extreme danger	Deadly
Fire (Flash Point)	Will not Burn	>200°F	100-200°F	73-100°F	<73°F
Reactivity	Stable	Unstable if heated	Violent Chemical change	Shock and Heat may detonate	May detonate

Notice to Reader: *Information presented in this MSDS has been compiled from sources considered to be dependable and is, to the best of Blaser Swisslube Inc. knowledge, accurate and reliable, but is not guaranteed to be so. The Data in this MSDS relates only to the specific material designated herein. Blaser Swisslube Inc. assumes no legal responsibility for the use or reliance upon these data. Since conditions of use are not under our control, Blaser Swisslube Inc. makes no warranty as to the results to be obtained in using this material and we must necessarily disclaim all liability with respect to the use of any material supplied by us.*

Attachment 6

Planta (1986) 168:571–578

Planta

Calcium acetate induces calcium uptake and formation of calcium-oxalate crystals in isolated leaflets of *Gleditsia triacanthos* L.

R. Borchert

Department of Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045, USA

Abstract. During treatment of isolated, peeled leaflets of *Gleditsia triacanthos* with 0.5–2 mM [^{45}Ca]acetate, saturation of the cell-wall free space with Ca^{2+} occurred within 10 min and was followed by a period of 6–10 h during which there was no significant Ca-uptake into the protoplast, but apoplastic Ca^{2+} was periodically released into the medium. Later, Ca^{2+} was absorbed for 3–4 d at rates of up to 2.2 μmol $Ca^{2+} \cdot h^{-1} \cdot (g\ FW)^{-1}$ to final concentrations of 350 μmol $Ca^{2+} \cdot (g\ FW)^{-1}$. The distribution of absorbed Ca^{2+} between cell wall, vacuole and Ca-oxalate crystals was determined during Ca-uptake. Wheras intact, cut leaflets deposited absorbed Ca^{2+} as Ca-oxalate in the crystal cells, peeled leaflets lacking crystal cells accumulated at least 40–50 μmol·$(g\ FW)^{-1}$ soluble Ca^{2+} before the absorbed Ca^{2+} was precipitated as Ca-oxalate. These observations indicate that the mechanisms for the continuous uptake of Ca^{2+}, the synthesis of oxalate and the precipitation of Ca^{2+} as Ca-oxalate are operational in the crystal cells of intact leaflets, but not in the mesophyll cells of peeled leaflets where they must be induced by exposure to Ca^{2+}. The precipitation of absorbed Ca^{2+} as Ca-oxalate by the crystal cells of isolated *Gleditsia* leaflets illustrates the role of these cells in the excretion of surplus Ca^{2+} which enters normal, attached leaves with the transpiration stream.

In addition to acetate, only Ca-lactate and Ca-carbonate lead to Ca-uptake, but at rates well below those observed with Ca-acetate. Other small organic anions (citrate, glycolate, glyoxalate, malate) and inorganic anions (chloride, nitrate, sulfate) did not permit Ca-uptake. Acetate-^{14}C was rapidly absorbed during Ca-uptake, but less than 20% was incorporated into Ca-oxalate; the rest remained mostly in the soluble fraction or was metabolized to CO_2. Acetate, as a permeable weak acid, may enable rapid Ca-uptake by stimulating proton extrusion at the plasmalemma and by serving as a counterion during Ca-accumulation in the vacuole, but is unlikely to function as the principal substrate for oxalate synthesis.

Key words: Acetate – Calcium uptake – Calcium oxalate crystals – Crystal-cell induction – *Gleditsia*.

Introduction

Calcium-oxalate crystals are common in cells and tissues of higher plants, yet the mechanisms involved in calcium accumulation and crystal formation, and the role of calcium excretion with respect to the mineral economy of the whole plant have received only little attention (see reviews by Arnott and Pautard 1970; Franceschi and Horner 1980).

In the course of the growing season, the leaflets of *Gleditsia triacanthos* L. (honey locust) accumulate large quantities of calcium-oxalate crystals in a highly specific temporal and spatial pattern (Borchert 1984). The time course and pattern of crystal formation in *Gleditsia* leaflets indicate that differentiation of crystal-forming cells (crystal cells or crystal idioblasts) might be induced by high concentrations of extracellular Ca^{2+} in the leaf tissues.

When isolated, peeled leaflets of *Gleditsia* seedlings are floated on 0.3–2 mM Ca-acetate, increasing numbers of Ca-oxalate crystals are deposited in the mesophyll in a characteristic, concentration-dependent, spatial pattern (Borchert 1985). Crystals are not induced by Ca-chloride or nitrate. Three phases were identified in the induction of crystals: an initial period of *adaptive aging*, during which Ca^{2+} is not required and crystal induction is not possible; a 48-h *induction period*, during which exposure to 1–2 mM Ca-acetate induces the differentiation of mesophyll cells into crystal cells; and *crystal growth*, which begins 72 h after the start of crystal-cell induction (Borchert 1985).

Cells of terrestrial plants, like those of all eucaryotic organisms, maintain very low cytoplasmic concentrations of Ca^{2+} (Marmé and Dieter 1983; Hepler and Wayne 1985). Elimination of surplus Ca^{2+}, which enters plants with the water absorbed by the roots and is left behind when water evaporates in transpiration, must therefore represent a major physiological problem for these plants. As inducible sinks for the orderly excretion of surplus Ca^{2+} in inert form, crystal cells may represent the solution of this problem and thus play a role in the plant's Ca-metabolism which has not been fully recognized in the past.

In the analysis of Ca-uptake during crystal induction described in this paper, it was found that acetate and to a lesser extent lactate and carbonate, but not other anions, facilitated the absorption and accumulation of relatively large quantities of Ca^{2+} by isolated, peeled *Gleditsia* leaflets.

Material and methods

Experimental material; microscopic observation of Ca-oxalate crystals. Seedlings of *Gleditsia* were grown and leaflets were peeled as described in Borchert (1985). For microscopic observation and microphotography in polarized light, leaflets were fixed, destained, and imbedded as also described in Borchert (1985).

Measurement of Ca-uptake. For short-term (24–48 h) measurements of Ca-uptake, peeled leaflets were aged at 25° C for 12 or 24 h under constant illumination (400 mmol·m^{-2}·s^{-1}; fluorescent F40PL Plant Light; General Electric, Cleveland, O., USA) on 0.1 mM Ca-acetate (40 leaflets per 10-cm-diameter Petri dish containing 15 ml solution). Normally, 10 leaflets per experimental treatment were used. Sets of 10 peeled leaflets weighed between 36 and 45 mg. The fresh weight of 10 peeled leaflets was assumed to be 40 mg in all calculations, because weighing peeled leaflets for each experiment was found to be impractical, and the leaflets varied not only with respect to size (fresh weight and leaf area), but also with respect to the fraction of leaf surface actually peeled. Leaflets were treated in small (6 cm diameter) disposable plastic Petri dishes containing 2 or 3 ml experimental solution with $10\cdot10^5$–$15\cdot10^5$ cpm·ml^{-1} ^{45}Ca (added as $CaCl_2$; Amersham Corporation, Arlington Heights, Ill., USA) and shaken at 100 rpm under continuous light (see above). Two 10-µl aliquots were pipeted at the indicated times onto 1-cm^2 pieces of filter paper, which were then inserted into 3-ml Nalgene Filmware tubes (Nalge Co., Rochester, N.Y, USA) containing 1 ml scintillation liquid (Scintiverse E; Fisher Scientific Co., Fairlawn, N.J., USA). Samples were counted in a liquid scintillation counter (Packard Tricarb; United Technologies Packard, Downers Grove, Ill., USA). Using a program written in BASIC, the following values were calculated on a microcomputer for each sampling time from the disappearance of ^{45}Ca from the experimental solutions: Ca-uptake as µM Ca^{2+}·(10 leaflets)$^{-1}$ and µM Ca^{2+}·(g FW)$^{-1}$ (equivalent to the $[Ca^{2+}]$ in mM in the tissue), rate of Ca-uptake as µmol Ca^{2+}·h^{-1}·(g FW)$^{-1}$, and percent uptake of ^{45}Ca in the original experiment solution. In each experiment, individual treatments were done in duplicate, and experiments were repeated at least three times. In important experiments, errors were calculated from three or four identical experimental treatments in one experiment.

For measurement of long-term Ca-uptake (3–6 d), 10 leaflets per experimental group were floated under constant illumination (see above) on 25–150 ml experimental solution in stoppered 125- or 250-ml Erlenmeyer flasks and stirred at 100 rpm with magnetic stirrers. Two 50-µl aliquots were taken at the indicated times, added to 1 ml scintillation liquid in 3-ml Nalgene Filmware tubes, counted, and evaluated as described above.

Uptake of [^{14}C]acetate. Methods for uptake of [^{14}C]acetate (Na-acetate; Amersham Corp.) were identical to those described above for Ca-uptake. To determine the release of $^{14}CO_2$ during long-term uptake of acetate, a 3-ml plastic cup containing 5 cm^2 filter paper soaked with 0.5 ml 20% KOH (w/v) was suspended in the air space of a tightly sealed (Parafilm plus rubber stopper) Erlenmeyer flask; at the end of the experiment, the filter paper was dried, placed into 5 ml scintillation liquid, and the radioactivity counted as described above.

Distribution of ^{45}Ca and [^{14}C]acetate in tissue fractions. After the determination of the uptake of Ca^{2+} or acetate, leaflets were washed for a minimum of 6 h in 10 ml of the washing solutions described in *Results*. Aliquots (2 ml) of the washes and, ultimately, the washed leaflets were added to 4 ml scintillation liquid and counted in 7-ml scintillation vials as described above. To verify the formation of Ca-oxalate crystals in the leaflets during long-term uptake experiments, groups of leaflets were treated like the corresponding experimental groups, but without radio isotope, and were examined microscopically as described in Borchert (1985).

Results

During the experimental induction of calcium oxalate crystals in isolated *Gleditsia* leaflets, crystal formation was observed to begin 3 d after exposure of the leaflets to the inductive Ca-solution (Borchert 1985). In the present study, uptake and accumulation of Ca^{2+} during the induction period preceding crystal formation has been analyzed, but the criterium for optimum induction remains crystal formation following induction.

Table 1. Calcium-oxalate-crystal formation in peeled *Gleditsia* leaflets as a function of Ca-concentration and total available Ca^{2+} in the experimental solution. Ten leaflets per treatment were floated for 6 d on variable volumes of Ca-acetate containing the indicated amounts and concentrations of Ca^{2+}. Scoring of crystal formation: 0–no crystals present; 1–few, scattered crystals; 2–many crystals along the veins, but few in the mesophyll; 3–crystals in up to 50% of mesophyll area; 4–mesophyll densely packed with crystals (see Fig. 1B)

$[Ca^{2+}]$	Total Ca^{2+} (µmol)			
	2.5	5	10	20
0.25 mM	0–1	1–2	1–2	
0.5 mM	0–1	2–3	3–4	4
1.0 mM		1–2	3–4	3–4
2.0 mM			3	3–4

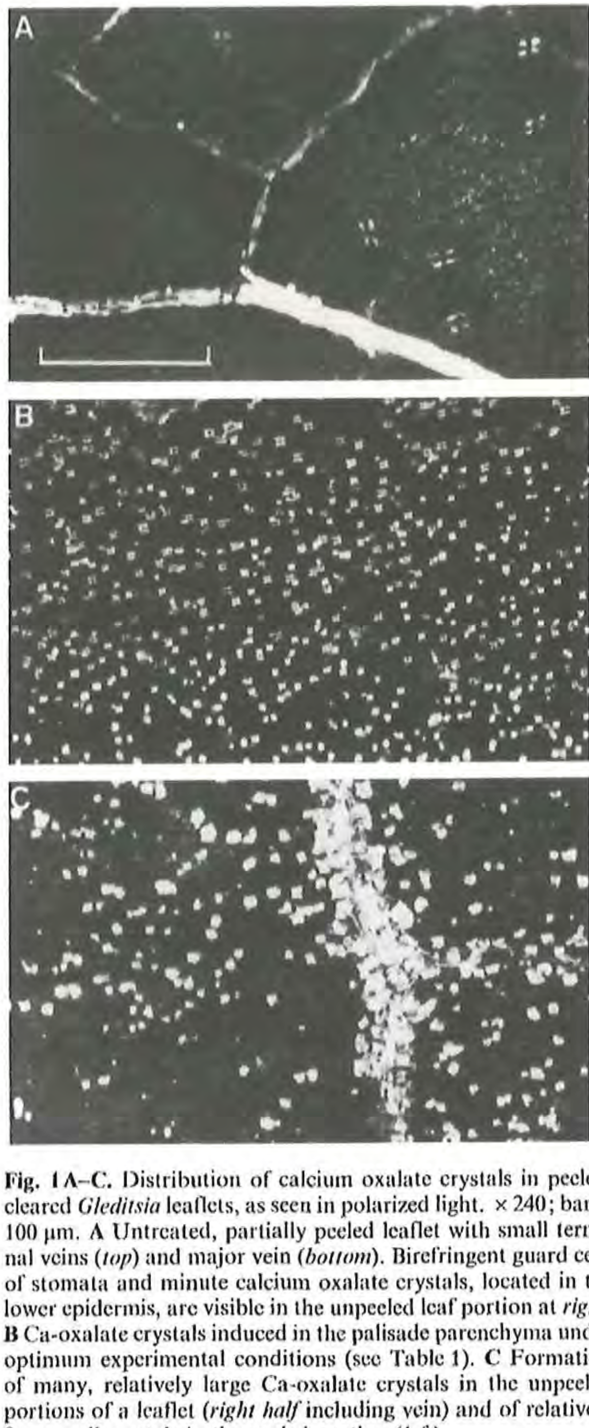


Fig. 1A–C. Distribution of calcium oxalate crystals in peeled, cleared *Gleditsia* leaflets, as seen in polarized light. × 240; bar = 100 μm. **A** Untreated, partially peeled leaflet with small terminal veins (*top*) and major vein (*bottom*). Birefringent guard cells of stomata and minute calcium oxalate crystals, located in the lower epidermis, are visible in the unpeeled leaf portion at *right*. **B** Ca-oxalate crystals induced in the palisade parenchyma under optimum experimental conditions (see Table 1). **C** Formation of many, relatively large Ca-oxalate crystals in the unpeeled portions of a leaflet (*right half* including vein) and of relatively few, small crystals in the peeled portion (*left*)

Optimization of crystal formation. Crystal formation on small, constant volumes of experimental solutions of variable $[Ca^{2+}]$ was observed to be irregular. Since crystal formation is likely to vary as a function of both $[Ca^{2+}]$ and total Ca^{2+} in the medium, crystal formation was determined using variable volumes of experimental solutions

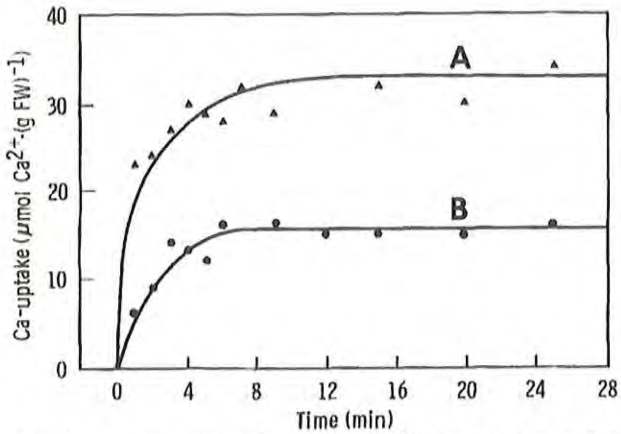


Fig. 2. Calcium uptake from 1 mM $[^{45}Ca]$acetate by peeled *Gleditsia* leaflets during the first 30 min of experimental treatment. *Curve A*, freshly peeled, 25-d-old leaflets; *curve B*, freshly peeled, 45-d-old leaflets. Ten leaflets were placed into 2 ml experimental solution, two 10-μl aliquots were taken at the indicated intervals, and Ca-uptake was calculated from the disappearance of ^{45}Ca from the experimental solution as described in *Material and methods*

(Table 1). Combinations of 0.5–2 mM Ca^{2+} and 2 μmol Ca^{2+} per leaflet (= 50 μmol Ca^{2+} · $(g\ FW)^{-1}$) were found to be optimal for crystal formation (Fig. 1A, B) and were therefore used in long-term Ca-uptake studies.

Uptake of Ca from Ca-acetate. In measurements of Ca-uptake by plant tissues, ionic binding of Ca^{2+} by the negatively charged cell-wall matrix must be distinguished from Ca-uptake into the protoplast (Macklon 1984). When placed on 1 mM Ca-acetate, peeled, aged or non-aged *Gleditsia* leaflets rapidly absorbed Ca^{2+} into the cell-wall free space (Fig. 2). Within 10–12 min $[Ca^{2+}]$ in the tissue reached 15–35 μmol·$(g\ FW)^{-1}$. Consistently, initial Ca-uptake by older leaflets (40–50 d old) was significantly lower than by young (22–27 d old) leaflets (Fig. 2). In numerous Ca-uptake experiments, $[Ca^{2+}]$ in young leaflets measured 30 or 60 min after initial exposure to ^{45}Ca was in the range of 20 to 35 μmol·$(g\ FW)^{-1}$ (sources of variability in the experiments are discussed in *Material and methods*), and only Ca-uptake above this range may therefore be considered as Ca-uptake into the protoplast.

Uptake of Ca from 0.5 and 2 mM Ca-acetate, measured at 12-h intervals, was rapid for 3 d and then began to level off (Fig. 3A: I, II). The maximum rate of Ca-uptake was 2.2 μmol $Ca^{2+}\cdot h^{-1}\cdot (g\ FW)^{-1}$ and the final $[Ca^{2+}]$ in the tissue was between 250 and 350 μmol·$(g\ FW)^{-1}$ in all experiments (compare Table 2).

To determine the distribution of absorbed Ca^{2+} in the leaf cells, the following washes were

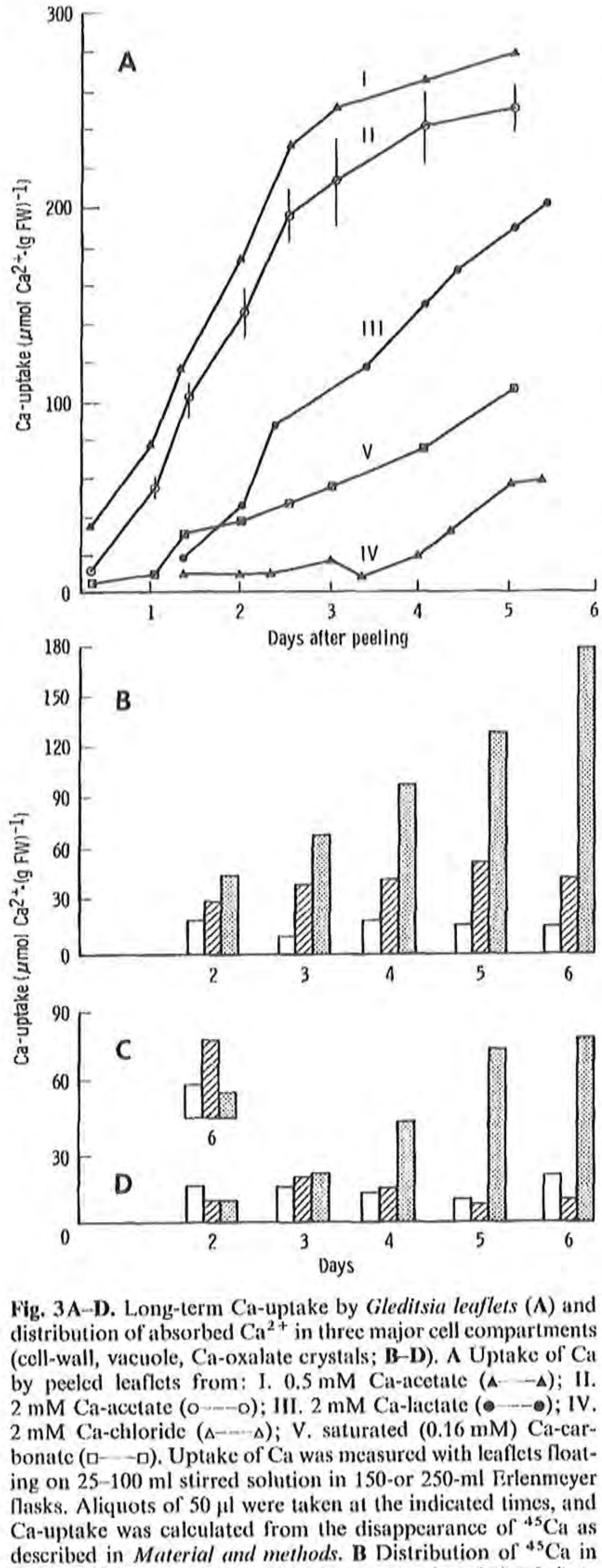


Fig. 3A–D. Long-term Ca-uptake by *Gleditsia leaflets* (A) and distribution of absorbed Ca^{2+} in three major cell compartments (cell-wall, vacuole, Ca-oxalate crystals; **B–D**). **A** Uptake of Ca by peeled leaflets from: I. 0.5 mM Ca-acetate (▲—▲); II. 2 mM Ca-acetate (○—○); III. 2 mM Ca-lactate (●—●); IV. 2 mM Ca-chloride (△—△); V. saturated (0.16 mM) Ca-carbonate (□—□). Uptake of Ca was measured with leaflets floating on 25–100 ml stirred solution in 150-or 250-ml Erlenmeyer flasks. Aliquots of 50 μl were taken at the indicated times, and Ca-uptake was calculated from the disappearance of ^{45}Ca as described in *Material and methods*. **B** Distribution of ^{45}Ca in major cell compartments after treatment of peeled *Gleditsia* leaflets with 2 mM Ca-acetate for 2–6 d. Leaflets were incubated on ^{45}Ca-solutions as described for **A** above, sampled at the indicated days, and washed in the following washes as described in *Material and methods*. *Left bars*: cell-wall-bound Ca^{2+}, washed out with 3 mM Na-EDTA. *Center bars*: soluble (mostly vacuolar) Ca^{2+}, released by 95% ethanol + 10 mM Ca-chloride. *Right bars*: insoluble Ca^{2+} (Ca-oxalate) released by 2 N HCl + 10 mM $CaCl_2$. After this wash, only minute amounts of Ca^{2+} remained in the leaflets. **C** Distribution of Ca in peeled leaflets treated with 2 mM Ca-chloride for 6 d. **D** Distribution of Ca in unpeeled leaflets with two parallel, longitudinal cuts, treated with 2 mM Ca-acetate

Table 2. Uptake of ^{45}Ca and ^{14}C-acetate by peeled *Gleditsia* leaflets during the formation of calcium-oxalate crystals. Data are the means of five replicates taken after 5 d incubation of 10 leaflets on 50 ml 0.5 mM Ca-acetate

	^{45}Ca	SD	^{14}C	SD
Solute uptake (μmol)	12.9	1.5	31.4	2.8
[Solute] in the tissue (μmol)·(g FW)$^{-1}$	323.3	36.6	776.4	75.0

used (Fig. 3B–D): (1) 3 mM Na_2-ethylenediaminetetraacetate (Na-EDTA), which effectively binds cell-wall-bound Ca^{2+}, but cannot penetrate the plasmalemma to chelate soluble Ca^{2+} within the protoplast; (2) 95% ethanol + 10 mM Ca-chloride (to displace ^{45}Ca) releases all soluble Ca^{2+} from the various cell compartments by rendering membranes permeable. In view of the quantities of absorbed Ca^{2+}, it is likely that most soluble Ca^{2+} is stored in the vacuole; (3) 2 N HCl dissolves Ca-oxalate crystals and thus releases Ca^{2+} insoluble in the ethanol wash. Between 83 and 96% of the previously absorbed ^{45}Ca was recovered in the washes in most experiments. Throughout the period of Ca-uptake by peeled leaflets, cell-wall-bound Ca^{2+} constituted the smallest and, as expected, rather constant fraction of absorbed Ca^{2+}; soluble Ca^{2+} increased moderately to 40–50 μmol Ca^{2+}·(g FW)$^{-1}$, but Ca^{2+} precipitated as Ca-oxalate increased dramatically with time (Fig. 3B).

During peeling of *Gleditsia* leaflets most crystal cells located in the bundle sheath of major veins are removed with the lower epidermis (Fig. 1A). The number of Ca-sinks in peeled leaflets is thus significantly reduced until new ones have formed during experimental induction of crystal cells (Borchert 1985). If Ca-uptake by unpeeled leaflets is made possible by means of longitudinal cuts with a razor blade rather than by peeling, the number of functional Ca-sinks should not be affected and cut leaflets should effectively convert absorbed Ca^{2+} to Ca-oxalate in the existing Ca-sinks. Compared with peeled leaflets, Ca-uptake by cut leaflets was relatively low, and the soluble fraction remained very small – usually below 15 μmol Ca^{2+}·

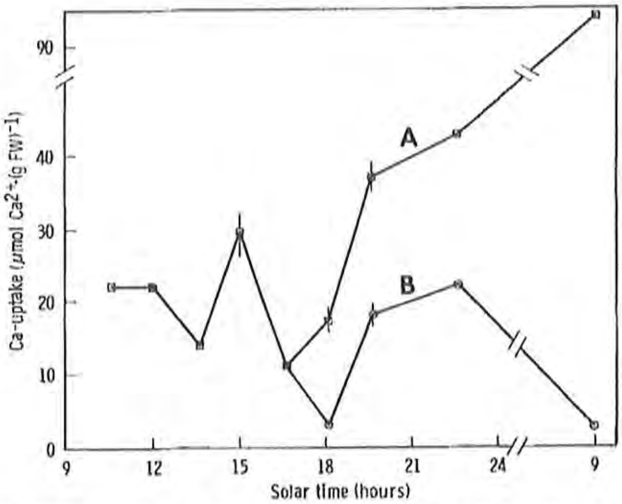


Fig. 4. Uptake of Ca by peeled *Gleditsia* leaflets from 2 mM [^{45}Ca]acetate between 24 and 48 h after peeling. *Curve A*, 2 mM Ca-acetate (□—□); *curve B*, 2 mM Ca-chloride (○—○). Leaflets were aged for 24 h on 0.1 mM Ca-acetate, then treated on 3 ml experimental solution per 10 leaflets. Two 10-µl aliquots were taken at the indicated times and Ca-uptake was calculated as described in *Material and methods*

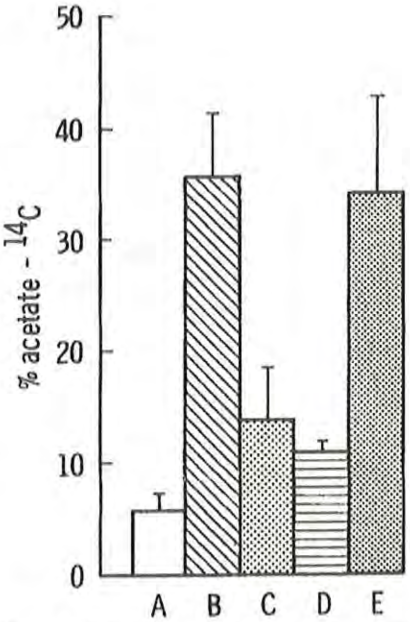


Fig. 5. Distribution of ^{14}C absorbed from 0.5 mM Ca-acetate in various fractions of *Gleditsia* leaflets after 5 d of experimental treatment. *Bars A–C*: acetate-^{14}C located in the cell-wall (*A*), in the soluble fraction of the protoplast (mostly vacuole; *B*), and in Ca-oxalate (*C*) (washes as described in Fig. 3B); *bar D*: ^{14}C remaining in the leaflets after the last wash; *bar E*, ^{14}C in CO_2 trapped by KOH in the airspace above the experimental solution

$(g\ FW)^{-1}$ – throughout the experiment (Fig. 3D). The absorbed Ca^{2+} was deposited mostly in existing crystal cells along the veins, where small Ca-oxalate crystals grew notably during the experimental treatment; only few crystal cells were newly induced in the palisade parenchyma.

The time course of long-term Ca-uptake as described in Fig. 3A indicates that Ca-uptake proceeds steadily at relatively high rates for several days. However, measurement of Ca-uptake by aged and non-aged leaflets at 1- or 2-h intervals showed that during the first 10 h of treatment with 1 or 2 mM Ca-acetate, periods of Ca-uptake alternated with periods during which a large fraction of the previously absorbed Ca^{2+} was released into the experimental solution (Fig. 4). When leaflets were aged for 24 h and treated with Ca-solutions in the morning, there was always a moderate release of Ca^{2+} between 12:00 and 14:00 h and an almost complete release of previously absorbed Ca^{2+} during the late afternoon (17:00–19:00 h) of the leaflets' first day of exposure to Ca^{2+}. Leaflets placed on Ca-acetate during the early afternoon showed only the second period of Ca-release. Essentially the same time course of Ca-absorption was observed with leaflets placed on 1 mM Ca-acetate right after peeling (data not shown). During the following days Ca-uptake from Ca-acetate proceeded at high, rather constant rates (Fig. 4A), but leaflets treated with Ca-chloride eventually released all previously absorbed Ca^{2+} into the medium (Fig. 4B).

The role of acetate in the induction of calcium-oxalate crystals. Enhancement of Ca-uptake by acetate (Fig. 3, Table 2) indicates that acetate may make Ca-uptake possible by serving as a counterion which permeates the plasmalemma in its protonated form, enters the vacuole, and balances the charges of Ca^{2+} accumulating in the vacuole. Acetate might also function as a substrate for oxalate synthesis, and acetate-^{14}C should then be incorporated preferentially into the acid-soluble Ca-oxalate fraction. In either case, 2 mol of acetate should be taken up for each mole of Ca^{2+} absorbed. A comparison between the uptake of ^{45}Ca and [^{14}C]acetate shows that acetate uptake during 5 d of treatment was approx. 25% higher than the predicted stoichiometry (Table 2).

The distribution of absorbed ^{45}Ca and acetate-^{14}C in the various tissue fractions was compared using the washes described above (Fig. 3B); in addition, retention of ^{14}C in the acid-washed leaflets and release of $^{14}CO_2$ into the gas phase of the experimental vessel was determined (Fig. 5). In contrast to the distribution of ^{45}Ca observed after treatment with Ca-acetate (Fig. 3B, 6 d), very little ^{14}C was found in the cell-wall fraction, but a major part remained in the soluble protoplast fraction; less than 20% ^{14}C was incorporated into Ca-oxa-

Table 3. Uptake of Ca by peeled *Gleditsia* leaflets from solutions of the Ca-salts of various organic and inorganic anions. Leaflets were aged for 24 h on 0.1 mM Ca-acetate, then placed on 3 ml 2 mM Ca-solutions adjusted to pH 6.8 with $Ca(OH)_2$. Uptake of ^{45}Ca was measured as described in *Material and methods*

	Experimental treatment	µmol $Ca^{2+} \cdot (g\ FW)^{-1}$ after ...		
		3 h	9 h	24 h
	Organic onions:			
A	Acetate	36	71	131
B	Lactate	39	47	53
C	Glycolate	14	14	22
D	Glyoxalate	15	14	17
E	Citrate	24	13	12
F	Malate	15	8	11
G	Ascorbate	25	20	13
	Inorganic anions:			
H	Chloride	26	10	0
I	Nitrate	15	5	0
J	Sulfate	12	4	0
K	*Ca-uptake by cell walls after 30 min*	20–28		

late, but substantial amounts were retained in the acid-washed leaflets (presumably in starch, suberin and other polymers synthesized during treatment) or metabolized to CO_2.

Effect of various anions on Ca-uptake. In earlier work it had been found that formation of Ca-oxalate crystals could be induced in peeled *Gleditsia* leaflets by Ca-acetate and Ca-carbonate, but not by inorganic salts such as Ca-chloride, nitrate or sulfate (Borchert 1985). The effect of various inorganic and small organic anions on Ca-uptake was therefore studied (Fig. 3, Table 3).

Uptake of significant amounts of Ca^{2+} during the crystal induction phase occurred, in addition to acetate, only with lactate and carbonate (Fig. 3A, III, V); rates of Ca-uptake from solutions of these latter salts were notably lower than from acetate, and with Ca-carbonate there was a distinct lag phase before any measurable Ca-uptake occurred (Fig. 3A, V). Both anions induced the formation of numerous Ca-oxalate crystals. No Ca^{2+} was absorbed from Ca-chloride during the first 4 d; the small amount of Ca^{2+} taken up during days 5 and 6 (Fig. 3A, IV) remained mostly in the soluble fraction and was not precipitated as Ca-oxalate (Fig. 3C).

During a 24-h treatment of aged, peeled leaflets with 2 mM Ca-salts of various small organic acids, substantial Ca-uptake was observed only with acetate and lactate, whereas virtually no Ca^{2+} was absorbed into the protoplast from solutions of other small organic Ca-salts (Table 3A–G); however, the leaflets retained most of the cell-wall-bound Ca^{2+} taken up initially (20–28 mM Ca^{2+}). Calcium propionate was also tested but found to be toxic to the leaflets. In treatments with inorganic Ca-salts (Table 3H–J) there was no Ca-uptake into the protoplast, and wall-bound Ca^{2+} was completely released within 24 h. If leaflets were transferred to 2 mM Ca-acetate after a 24-h treatment with the above organic or inorganic salts, Ca-uptake proceded normally on Ca-acetate.

Discussion

Phases of Ca-uptake. The following three phases, differing in the kinetics of Ca-uptake, could be distinguished during Ca-uptake by peeled leaflets from 0.5–2 mM Ca-acetate:

(1) Uptake of Ca^{2+} into the cell-wall free space. Like other plant tissues, young, peeled *Gleditsia* leaflets placed on Ca-solutions rapidly absorbed 20–35 µmol $Ca^{2+} \cdot (g\ FW)^{-1}$ into the cell-wall free space (Fig. 2; Marré et al., 1982; Demarty et al. 1984). Similar concentrations of negative charges in the cell-wall have been found in other plant tissues (12 and 16 µmol$\cdot (g\ FW)^{-1}$ for beet root tissue and *Atriplex* leaf slices, respectively; Lüttge and Higinbotham 1979, p. 94). During the saturation of cell walls with Ca^{2+}, protons were displaced and released into the medium, as indicated by a rapid decline in pH from 6.15 to 5.5 during treatment with unbuffered 1 mM Ca-chloride (data not shown).

(2) Induction of the Ca-uptake mechanism. In other plant tissues, short-term, rapid cation uptake into the cell-wall free space is normally followed immediately by a slower, but continuous, active cation transport into the protoplast (Lüttge and Higinbotham 1979, p. 93; Marré et al. 1982). The kinetics of Ca-uptake into peeled *Gleditsia* leaflets differs in two respects from the pattern observed for cation uptake in other plant tissues: (i) for several hours after the initial saturation of the cell-wall free space with Ca^{2+} there is no measurable uptake of Ca^{2+} into the protoplast, and (ii) Ca^{2+} absorbed into the cell-wall is released periodically during the first 8–12 h of treatment of freshly peeled or aged leaflets with 1–2 mM Ca-solutions (Fig. 4). These observations indicate that certain components of the mechanism involved in the subsequent long-term uptake of Ca^{2+} into the protoplast (Fig. 3) and its storage in the vacuole are initially not functional, but are induced by the exposure of the leaflets to 1–2 mM Ca^{2+}. Most lik-

ely, Ca^{2+} released into the medium is displaced from the cell wall by H^+ extruded into the cell-wall space by proton pumps located on the plasmalemma (Lüttge and Higinbotham 1979, p. 130; Sze 1985; Romani et al. 1985). In numerous experiments, this release of Ca^{2+} occurred during the same periods of solar time (12:00–14:00 and 18:00–20:00); Fig. 4), but at different time intervals after the initial exposure of the leaflets to Ca^{2+}. The periodic activity of proton pumps thus appears not to be triggered simply by the initial exposure to Ca^{2+}, but might be also related to a circadian rhythmicity of ion transport as it is manifest in the nyctinastic movements of *Gleditsia* seedling leaflets and leaves of other leguminous trees (for a review, see Satter and Galston 1981).

(3) Continuous, long-term Ca-uptake. After the mechanism for Ca-uptake has become functional, isolated, peeled, illuminated *Gleditsia* leaflets are capable of absorbing Ca^{2+} at relatively high rates and of concentrating the absorbed Ca^{2+} more than 500-fold. These observations are in contrast with the widely held notion, based mostly on studies of Ca-uptake by isolated roots (Macklon 1984), that plant cells absorb Ca^{2+} only slowly and in small quantities.

Precipitation of absorbed Ca^{2+} as Ca-oxalate. If crystal cells are efficient sinks for surplus soluble Ca^{2+} as postulated in the *Introduction*, then Ca^{2+} taken up during experimental treatment of plant tissues containing crystal cells should be rapidly precipitated in these cells as Ca-oxalate. In agreement with this prediction, Ca^{2+} absorbed by isolated, unpeeled, but longitudinally cut *Gleditsia* leaflets was mostly precipitated as Ca-oxalate, and the content in cell-wall-bound and soluble (vacuolar) Ca^{2+} remained low throughout the treatment period (Fig. 3D). In peeled *Gleditsia* leaflets, from which crystal cells have been removed during peeling (Fig. 1A), Ca^{2+} had been found earlier to accumulate in soluble form for 3 d before Ca-oxalate crystals became visible (Borchert 1985). Contrary to these observations, during treatment of peeled leaflets with Ca-acetate, there was a progressive accumulation of Ca-oxalate well before new crystals had appeared (Fig. 3B, 2–4 d), but leaflets also accumulated 40–50 $\mu mol \cdot (g\ FW)^{-1}$ soluble Ca^{2+} (Fig. 3B). These data reflect the fact that actually in "peeled" leaflets variable portions (10–30%) remain unpeeled and thus contain crystal cells capable of precipitating absorbed Ca^{2+} (Fig. 1C). The observed concentrations of soluble, most likely vacuolar, Ca^{2+} are much higher than values reported for other plant tissues such as onion roots (2 mM; Macklon 1984) and various leaves (2–22 mM; Jeschke 1976).

The accumulation of substantial quantities of soluble vacuolar Ca^{2+} during Ca-uptake into leaf tissues lacking functional Ca-sinks in the form of crystal cells implies that oxalate is not available in these tissues to precipitate Ca^{2+} and that oxalate synthesis may be confined to functional crystal cells. Also, it is unlikely that acetate enhances Ca-uptake by functioning as a specific substrate for oxalate biosynthesis, because the period of maximum uptake of Ca^{2+} and acetate preceded maximum oxalate synthesis by a day or two (Fig. 3) and less than 20% of the absorbed acetate-^{14}C was incorporated into oxalate (Fig. 5).

The role of anions in Ca-uptake. The accumulation of large quantities of soluble Ca^{2+} in the vacuoles of mesophyll cells must be accompanied by the uptake of counterions balancing the positive charges of Ca^{2+}. The marked enhancement of Ca-uptake by acetate compared with other anions (Table 3), indicates that acetate may stimulate Ca-uptake because of its nature as a permeant, weak acid. In several recent studies (Brummer et al. 1984; Hager and Moser 1985; Romani et al. 1985) the uptake of weak permeant acids or their esters was observed to cause hyperpolarization of the plasmamembrane and an increase in K^+-uptake, presumably by stimulating the plasmalemma proton pumps through lowering the cytoplasmic pH. Also, the uptake of divalent cations (not including Ca^{2+}) followed an electrochemical gradient and was balanced by the efflux of cations, mostly protons (Marré et al. 1982). Like other weak acids, acetate is likely to stimulate plasmalemma H^+-transfer ATPases which establish an electrochemical gradient enhancing entry of Ca^{2+} into the cytosol, but acetate may also serve as a readily available counterion balancing the charges of Ca^{2+} accumulating in the vacuole or as a substrate for the synthesis of malate, the counterion commonly produced by plant cells during cation accumulation (Lüttge and Higinbotham 1979, p. 136).

Like acetate, carbonate induces optimum crystal formation and stimulates long-term Ca-uptake (Fig. 3A), probably by serving as a substrate for the synthesis of malate. Lactate should enhance Ca-uptake for the same reasons as acetate, although to a lesser extent because of its larger size and less lipophilic character. Dicarboxylic acids such as malate and citrate are not fully protonated at biological pH and thus, as charged molecules, cross the plasmalemma only slowly. For example, citrate (3 mM, pH 4.5) acidified the cytosol of maize coleoptile cells noticeably less than acetate (Brummer et al. 1984). Glycolate, glyoxalate and ascorbate, selected for testing because they are known precursors in the biosynthesis of oxalate, were likewise unable to support Ca-uptake (Table 3).

The complete release of cell-wall-bound Ca^{2+} into the medium during treatment with inorganic anions (Fig. 4B; Table 2 H–K) must reflect the release of protons into the cell wall, as it has been frequently observed during uptake of surplus cations (Lüttge and Higinbotham 1979, p. 137). However, in my study, proton release was not preceded or accompanied by a measurable Ca-uptake into the protoplast and occurred in the presence of small, permeable anions, indicating other causes for the inferred proton release than proton/cation exchange. Several divalent cations (Co^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}) were found to be absorbed from a 2 mM sulfate solution into excised corn roots, but Ca^{2+} was not absorbed (Marré et al. 1982), just as it was not taken up by peeled *Gleditsia* leaflets from Ca-sulfate (Table 3). The observed resumption of rapid Ca-uptake upon transfer of leaflets from sulfate and other non-stimulating anions to Ca-acetate indicates that these anions are not toxic to the tissue, but merely fail to facilitate Ca-uptake; this explains the earlier observation that these anions, unlike acetate and carbonate, do not induce the formation of crystal cells in isolated *Gleditsia* leaflets (Borchert 1985).

The role and differentiation of crystal cells. The formation of Ca-oxalate crystals in plant tissues has been studied for many years (for a review, see Franceschi and Horner 1980), but information concerning the function of these crystals remains scarce. In this study it has been demonstrated that crystal cells act as Ca-sinks and enable plant tissues to deposit surplus Ca^{2+} absorbed under experimental – and, implicitly, natural – conditions as Ca-oxalate and to maintain relatively low concentrations of soluble Ca^{2+} in the apoplast and vacuoles of the tissue. If the capacity of existing Ca-sinks is insufficient to precipitate most apoplastic Ca^{2+}, the tissue content in soluble Ca^{2+} will rise and new crystal cells are induced (Fig. 3C; Borchert 1985). The preliminary evidence presented here indicates that differentiation of crystal cells involves the induction of mechanisms for the transport of Ca^{2+} across plasmalemma and tonoplast into the vacuole, the synthesis of organic counterions required for storage of soluble Ca^{2+} in the vacuole, and, lastly, the induction of oxalate synthesis. Both Ca-uptake and the synthesis of organic counterions and oxalate depend on an adequate supply of light energy and photosynthetic products. It should thus come to no surprise that uptake and precipitation of relatively large quantities of Ca^{2+} is possible in isolated, illuminated leaflets, but apparently not in non-photosynthetic organs such as roots (Macklon 1984).

The reliable technical help by Russell Beardall and support by the University of Kansas General Research Fund and a Biomedical Support Grant are gratefully acknowledged.

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Received 13 February; accepted 21 April 1986